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PHLOEM OF WHITE PINE AND OTHER CONIFEROUS SPECIES

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(WITH FORTY-SEVEN FIGURES)

Introduction

The phloem of the gymnosperms differs in many details from that of the angiosperms. Certain structural features of the long fusiform elements found in conifers do not fit the classical picture of the sieve tube as a perforate element, adapted to rapid longitudinal conduction. While plasmodesmata in the sieve plates of many monocotyledons and certain herbaceous dicotyledons are extremely fine, approximating in diameter those of the primary pit areas of phloem and cortical parenchyma, the protoplasmic connections of the gymnosperms are even more tenuous. It is difficult to reconcile their structure with the concept of tubular connections developed from studies on vines and other specialized angiosperms. A detailed description of the phloem of white pine, *Pinus strobus* L., and of other coniferous species should clarify the structural relation of these plasmodesmata to their role in conduction. In addition, it should provide information of general morphological interest.

Material and methods

In addition to *Pinus strobus*, which was used in developmental studies, the following species were investigated:

Abietineae	Cupressineae
Pseudotsuga taxifolia	Thuja plicata
Tsuga canadensis	Juniperus virginiana
Picea glauca	Chamaecyparis obtusa
Picea canadensis	
Abies concolor	Araucarineae
Pinus rigida	Araucaria sp.
Pinus densiflora	Agathis sp.
Pinus parviflora	
Pinus resinosa	
Pinus monticola	
Taxodineae	Taxineae
Taxodium distichum	Taxus canadensis
Sequoia gigantea	Taxus cuspidata
Sequoia sempervirens	

Differentiating and functioning phloem tissues are highly hydrated and hence soft and plastic. To avoid shrinkage and distortion resulting from the use of alcohol and drastic killing agents, living tissue was examined wherever possible. In removing the cambium and phloem from a tree and in sectioning and studying the living tissue, the methods of BAILEY (2) and of BAILEY and ZIRKLE (3) were followed.

For studies of sieve tube contents, tangential or radial sections 30–100 μ thick were cut from blocks of living tissue. Owing to the transparency of the untreated cambium and young phloem, details of structure within the sieve tubes may be seen, since living cells often occur close to the surface of the section. Cambial initials and young sieve elements in such sections appear to be but slightly injured, if at all, the cytoplasm continuing to stream normally for from several hours to many days, depending on external environmental factors. In many cases the sections of white pine were cut and placed under observation within 15 minutes after removal of the tissue from the living tree.

During 1932, blocks of white pine were collected at intervals of two or three weeks throughout the year, and the extent of callus development in the sieve pits was studied both before and after staining with water soluble anilin blue. These results were checked

during 1933 and part of the growing season of 1935. The nature of the sieve tube contents was studied both in unstained cells and after vital staining of the vacuoles. Plasmolysis tests were conducted on living sections stained with neutral red. Following examination in the living condition, many of the sections were killed in IKI solution, swelled in IKI in 5 per cent sulphuric acid, stained with water soluble anilin blue, and mounted in a medium containing glycerin and zinc chloride (**5**). For sieve pit structure, dilute neutral red followed by NEBEL'S (**20**) callus stain was also used.

For permanent records, small blocks of the outer wood, cambium, and inner bark were killed in Flemming's strong fixative or in boiling water, and sectioned after imbedding in paraffin by the butyl alcohol method. Although useful as a record, such sections, in comparison with the untreated fresh material, present only a caricature of the living cambium and of sieve tube contents and walls.

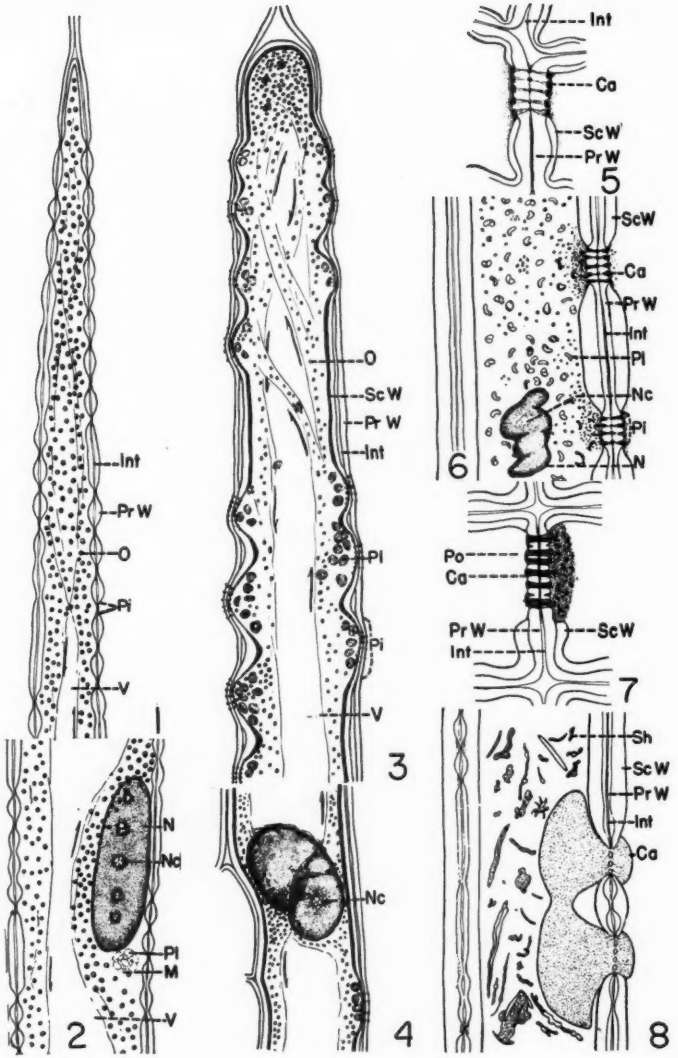
Observations

PHLOEM ONTOGENY IN WHITE PINE

INITIATION OF SIEVE TUBES.—Sieve tubes are of cambiform outline in tangential sections and, unlike tracheids, undergo little or no additional lengthening at the tips from the time of their derivation from the cambium to maturity. In transverse section they are usually more or less rectangular (fig. 9), having a tangential diameter approximately that of the cambial initials. The radial diameter is several times that of the initials. The length of cambial initials and sieve tubes is about one hundred times their tangential diameter (**2**, p. 659, fig. 15).

The wall of the cambial initial of white pine has been described and figured by KERR and BAILEY (**17**). There are essentially three layers present, two primary walls containing cellulose as well as polyuronides, and an intercellular substance composed of polyuronides. Numerous primordial pits occur in the radial walls of these initials (fig. 1).

The contents of the cambial initials have previously been described and figured (**1**, **2**). The streaming cytoplasm of the cambial initial commonly contains a single large vacuole. In the winter numerous conspicuous oil globules (fig. 10) are present in the slowly



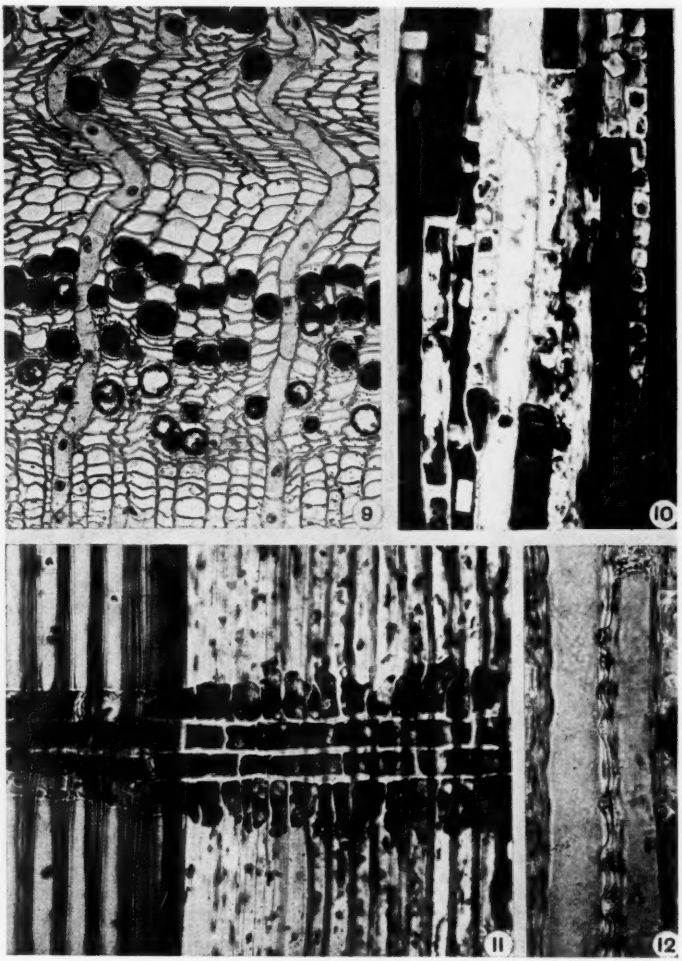
FIGS. 1–8.—White pine. (*Ca*, callus; *Int*, intercellular substance; *M*, mitochondria; *N*, nucleus; *Nc*, nucleolus; *O*, oil globule; *Pi*, pit; *Pl*, plastid; *Po*, sieve pore (callus filled); *PrW*, primary wall; *ScW*, secondary wall; *Sh*, shredlike body; *V*, vacuole.) Fig. 1, cambial initial, terminal portion. Fig. 2, median portion. Fig. 3, young sieve tube, terminal portion. Fig. 4, median portion showing crumpled nucleus. Fig. 5, callus initiation in pit. Fig. 6, callus formation in pits. Fig. 7, callus rods complete. Fig. 8, definitive callus and shredded bodies in senile sieve tube.

streaming, thick, viscous cytoplasm; but during the late spring and summer the size of the oil globules is reduced, and the layer of cytoplasm becomes thinner and streams more rapidly. Plastids (fig. 2*Pl*) and mitochondria (fig. 2*M*) occur in the cytoplasm but are often difficult to distinguish, owing to fluctuations in the opacity of the cytoplasm. The nucleus (fig. 2*N*) is a long, spindle-shaped body lying against one wall near the middle of the cell. It is rather densely granular and contains several nucleoli (fig. 2*Nc*) (1).

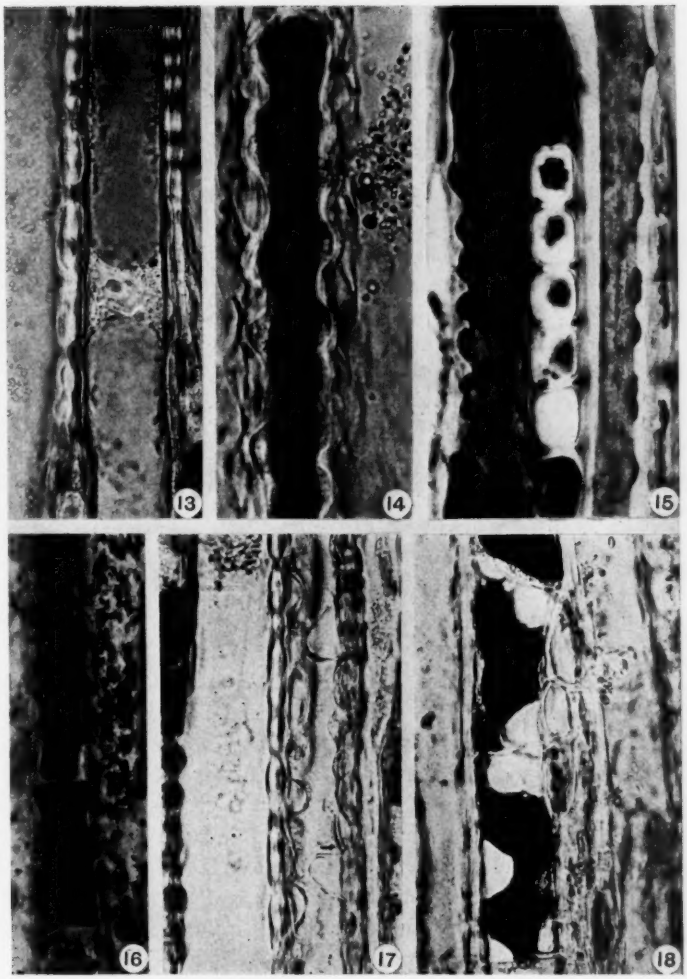
In Massachusetts, one of the first indications of growth in the spring is the enlargement of sieve tubes which failed to complete their differentiation during the preceding fall. Cambial cell division is relatively sluggish during March but is accelerated during April and May, resulting in the rapid formation of new xylem and phloem. Growth continues through the summer and fall at a diminishing rate, and sieve tubes initiated in late autumn may fail to complete differentiation until the following spring. Although young developing sieve tubes may at times be found in late November, the process of differentiation does not appear to be continuous throughout the winter. Given milder temperatures, sieve tube differentiation might proceed throughout the winter. This may account for the discrepancy between the writers' observations and those of STRASBURGER (21), who claimed that there are more differentiating stages in winter than during the growing season.

Indications of annual rings in the phloem of white pine (fig. 9) and of other Abietineae are an aid in studying the seasonal sieve tube increment. Sieve tubes which differentiate during the cooler, moister part of the year have slightly thinner walls and larger lumina than those found later, and the most conspicuous zone of parenchyma cells is formed in late spring in white pine (figs. 9, 10).

The young sieve tubes may be distinguished readily from young tracheids in tangential section by the following characteristics. The primary walls of the young sieve tubes are relatively thick, whereas those of tracheids are thinner. The plastids of the tracheids contain spindle-shaped bodies which stain blue-green in crystal violet and are distributed more or less evenly in the cytoplasm. The sieve tube plastids are located largely in the sieve areas (figs. 13, 14) and around the nucleus (fig. 13). They contain one to several granules at first,



FIGS. 9–12.—White pine. Fig. 9, transverse section of young phloem; ×200. Fig. 10, longitudinal tangential section of mature phloem showing tanniniferous parenchyma, starch-bearing parenchyma, rays, and crystals; ×250. Fig. 11, ray in longitudinal radial section; xylem on left, phloem on right; ×250. Fig. 12, young sieve tube in bulging stage (right) and mature sieve tube (left); protoplasm with plastids at top of young element; living tissue with neutral red staining; ×500.



FIGS. 13–18.—White pine. Fig. 13, young sieve tube in bulging stage showing protoplasmic bridge separating two vacuoles; living tissue with neutral red staining; ×500. Fig. 14, end of young sieve tube in bulging stage showing neutral red stained vacuole, plastids in cytoplasm of pit areas, and starch grains in neighboring mature tube on right; ×1000. Fig. 15, sieve tubes in material killed in IKI and stained with anilin blue; young sieve tube in bulging stage showing accumulation of cytoplasm in pit areas on left of ray, and more mature sieve tube with fibroid cytoplasm on right; ×500. Fig. 16, tanniniferous phloem parenchyma cells in bulging stage; fixed material; ×500. Fig. 17, plasmolyzed young sieve tube; ×500. Fig. 18, plasmolyzed phloem parenchyma with sieve tubes unaffected; ×500.

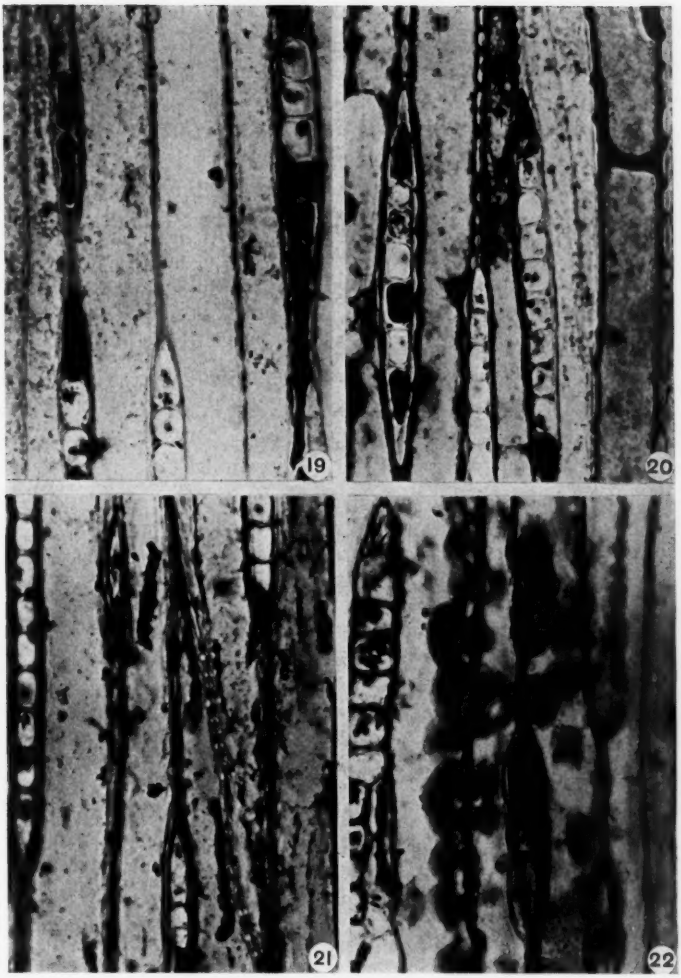
and later a single shell-shaped body which stains dark purple-blue in crystal violet. The younger sieve tube plastids are often covered with oil globules.

DETAILS OF SIEVE TUBE DIFFERENTIATION.—The early stages of sieve tube differentiation merge into one another but can be described in two steps. The wall and pit areas at first show little change except for a slight thinning down of the radial walls (fig. 19), especially during active growth in the spring.

In the spring, summer, and fall, the innermost sieve tubes (figs. 3, 4) have a layer of rapidly streaming, non-viscous protoplasm containing a large vacuole which stains orange in neutral red (fig. 14); whereas the vacuoles of the cambial initials stain magenta red. Imbedded in the cytoplasm, usually around the nucleus and in the pit areas, are the plastids (figs. 3, 4, 6, 13, 14). The usually roundish (fig. 13) or somewhat folded nucleus (figs. 4, 38) in such cells is more transparent than in the cambial initials, and has one to several nucleoli (see also 1, fig. 37). As differentiation proceeds, the nucleus swells slightly and becomes less elongate; the number of nucleoli is reduced. The oil globules become smaller and the cytoplasm streams more rapidly. Numerous leucoplasts may be observed at times in sieve tubes in this stage of development. These are plastic amoeboid bodies which change shape continually in the rapidly streaming protoplasm. They are found in cells which lack plastids with granules or have only a few such plastids.

TURGOR EXPANSION.—In the next stage (figs. 3, 4) cell enlargement occurs. The cytoplasm is in more active cyclosis, and there is evidence of internal pressures in many cells. The membrane of the pit areas often bulges outward (figs. 3, 12, 13, 14, 15), the pit enlarges, and its membrane becomes still thinner, owing to stretching. When two pits are close together, the wall between them often becomes rather thick at this stage, even before there is any evidence of secondary thickening (fig. 23). Slight thickening of the wall, due to apposition of secondary layers, may also be observed at times (figs. 3, 24, 26).

At this stage in sieve tube development, the parietal layer of cytoplasm becomes progressively thinner (figs. 12, 13). It streams around the cell in a steep spiral until it reaches the tip, where it



FIGS. 19–22.—White pine. Fig. 19, young sieve tubes and ray cells showing primary walls. Fig. 20, slightly older stage; tube on right shows some bulging; cytoplasm assuming parietal position and becoming fibroid and heavily staining; pitting to albuminous cells prominent. Fig. 21, slightly older stage of sieve tubes; highly fibroid cytoplasm torn in killing process, starch grains released into lumina. Diagonal wall between sister cells of recently divided initial shown in its primary condition. Fig. 22, definitive callus in senile phloem; all ×350.

mills around and returns down the opposite side of the cell without apparently passing into the pit areas, which show only a slight Brownian movement of their contents.

The large numbers of plastids are mostly clustered around the nucleus (figs. 4, 12, 13) or in the pit areas (figs. 12, 14). At first each plastid contains several dark granules, and later the typical shell-shaped body which gives a pale purple or red color in iodine-potassium-iodide. The reaction has already been referred to by STRASBURGER (**21**), HILL (**16**), and others. The "starch grains" in mature sieve tubes of many plants give this same reddish color in iodine-potassium-iodide (**4**), and since in polarized light they are optically birefringent, it seems safe to assume that they are composed of starch or some related carbohydrate.

The nucleus is usually roundish, nearly filling the lumen at the middle of the cell (fig. 13). Unlike the cambial nucleus, it usually divides the vacuole in two. There are one to several nucleoli, and the nucleus often appears to be crumpled (see also **1**, fig. 37).

Up to this stage of differentiation, the sieve tubes are capable of being plasmolyzed (fig. 17). The vacuoles take up neutral red from dilute buffer solutions relatively rapidly (figs. 12, 13, 14), and the cytoplasm continues to stream. These vacuoles are of the type B of BAILEY and ZIRKLE (**3**).

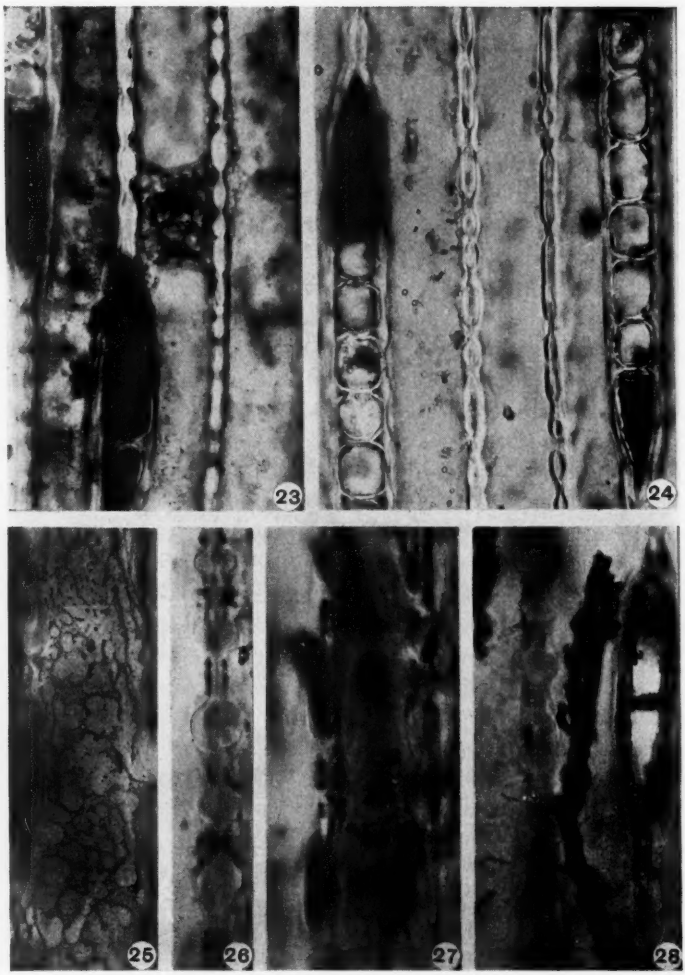
The bulging type of enlargement just described also occurs in young tracheids and parenchyma cells (fig. 16), and indicates that growth is a concomitant of high turgor pressure.

SIEVE TUBE MATURATION.—With the attainment of mature dimensions, the sieve tubes undergo changes of profound importance from both morphological and physiological standpoints. The nucleus of each cell, commonly located midway between the ends in a protoplasmic bridge dividing the lumen into two large vacuoles (fig. 13), expands and becomes less dense, often rumples (fig. 4), and finally fragments (fig. 23) and disintegrates, leaving no visible remnants. Attending this nuclear disintegration, the cytoplasm assumes a truly parietal position, as illustrated in the cell on the right of figure 23, passes through a stage when it becomes reticulate (fig. 25), and then fibroid (figs. 27, 28, 29). Finally assuming a truly parietal position, it loses its affinity for plasma stains and becomes

so thin and transparent that it is detected only with difficulty. Meanwhile the cell loses its ability to accumulate vital stains and can no longer be plasmolyzed. Figure 17 shows, at the right of center, a plasmolyzed young sieve tube, and at the left a mature element that has failed to respond to the hypertonic sucrose solution. Figure 18 shows mature phloem with plasmolyzed parenchyma cells of the A-vacuole type and two mature sieve tubes that are unaffected. In mature sieve tubes no vacuole (in the usual sense of the word) may be found, the interface between cytoplasm and the sap within the lumen being almost indistinguishable. The plastids break down, and their granules may be found later in the vacuolar sap, violently agitated by Brownian movement (fig. 6).

During this same ontogenetic period, the thin pit-closing membranes of the young sieve tube wall (fig. 24) thicken (figs. 26, 27), and the protoplasmic connections, demonstrable up to this time only by special methods involving severe swelling and dense staining (**5**), become visible by the water blue, iodine method (figs. 27, 28, 29). Careful study, using critical illumination and the best of optical equipment (Zeiss pancratic condenser; apochromatic 60×, 1.4 NA objective; and 15× Mobimi paired oculars), indicates that this increase in visibility is not due to any great increase in diameter of the threads but to the fact that they acquire a strong affinity for dyes and stain intensely. Furthermore, there is no evidence that they are "bored" out or transformed to "slime," as suggested by HILL (**16**). They later lose much of this affinity for dyes, just as does the parietal layer, and they are lost, together with the parietal layer, by dissolution when the element ceases to function. If the term slime is to be limited to the densely staining, finely particulate or colloidal material in the sieve tube lumen that results from disintegration of the nucleus, and in some species of slime bodies, then this material can be shown to be inclosed within the parietal protoplasm and to be distinct and separable from it. The slime is transitory in nature and soon disappears from the sieve tube, while the parietal cytoplasm, and the connecting threads of the sieve areas that are continuous with it, remain and can be demonstrated until lost at death of the cell.

Two other notable changes occur during this critical period in



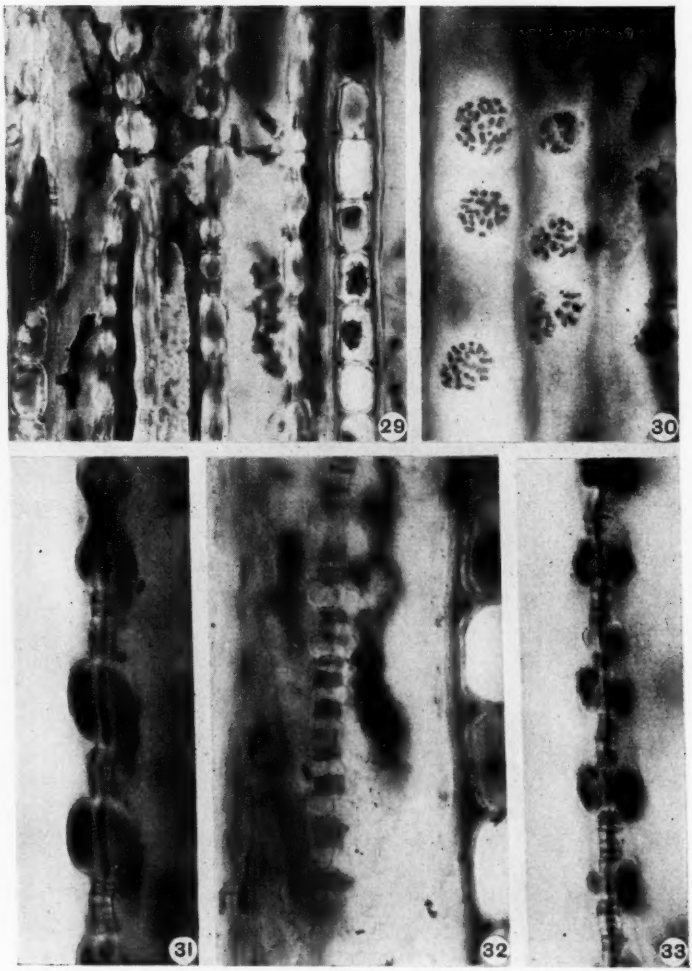
FIGS. 23–28.—White pine. Fig. 23, nuclear disintegration in young sieve tube; ×500. Fig. 24, first stages of secondary wall formation with plastids still present in cytoplasm; ×500. Fig. 25, sieve tube cytoplasm entering fibroid condition and increasing in staining affinity; ×1000. Fig. 26, common wall between two young sieve tubes; pit-closing membranes thickening and callus formation just starting; protoplasmic connections visible under microscope; ×875. Fig. 27, callus formation comparable with fig. 6. Note stringy condition of cytoplasm; ×1000. Fig. 28, threads in wall more clearly visible, although callus rods not completed; ×875.

sieve tube ontogeny. Attending the denaturing of the cytoplasm that occurs when the nucleus is lost, each individual thread (or small group of threads) traversing the sieve tube wall is surrounded by a callus cylinder or "rod." This material appears in small areas surrounding the protoplasmic threads of the sieve pits (figs. 5, 26), and increases inward (figs. 6, 27) until the cylinders converge at the middle lamella (figs. 7, 31, 32, 33). Figure 30 shows the ends of these rods as seen in radial longitudinal section. Figures 5, 6, and 7 illustrate successive stages in callus formation, from the initiation of rods to their completion.

The other important change taking place during sieve tube maturation is the development of secondary walls. This phenomenon is apparently restricted, among the species studied, to the Abietineae—the sieve tubes of Araucarineae, Taxodineae, Cupressineae, and Taxineae showing no evidence of a secondary wall. Figures 5, 6, 7, and 8 diagram this secondary thickening. Figure 23 shows the primary walls of young sieve tubes, figure 24 shows the beginnings of secondary thickening, and figures 26 and 28 show further development. In figures 27, 31, and 33 the walls were swelled somewhat with 5 per cent H_2SO_4 and stained with IKI. With this treatment they turned a light lavender and photographed darker. Note also the swelling and dense staining of the intercellular substance brought about by iodine and anilin blue in figures 27, 31, and 33. The median nodules described by HILL and others were black after this treatment.

When viewed in radial longitudinal section, the pits resulting from secondary wall formation are elliptical and oriented at about 90° in the walls of adjacent cells. This results in the peculiar skewed formation of the secondary thickenings in figure 31, which was cut somewhat off center as compared with figure 33 that more nearly bisected the pits. The Abietineae form the only group in which secondary wall thickening has been shown to occur in sieve tubes.

Radial and tangential sections of white pine phloem, when viewed in polarized light between crossed Nicols, showed a striated structure of the secondary walls from the latest streaming stages on. There was no evidence of the loss of this layer in the older sieve tubes. Previous to cell enlargement, the writers found no evidence



FIGS. 29–33.—White pine. Fig. 29, phloem showing extreme fibroid condition of cytoplasm and its intimate relation with sieve pits; ×500. Fig. 30, sieve pits in longitudinal radial view showing ends of callus cylinders; ×1000. Fig. 31, sieve pits in wall swelled with 5% H_2SO_4, showing connecting threads and secondary wall structure; ×1000. Fig. 32, similar view but with less swelling; callus rods complete as in fig. 7; ×1000. Fig. 33, view like fig. 31, showing median nodules, callus cylinders, and swelled secondary wall thickening; ×1000.

of spiral structure in the cambial or young sieve tube walls of this species. After swelling in 30 per cent sodium hydroxide and staining in iron-alum haematoxylin, spiral striations corresponding to those seen in polarized light were distinct in the radial and tangential walls of the older sieve tubes.

The following test for spiral arrangement was positive for the secondary layers of the sieve tube walls of white pine. Starting with transverse sections of the phloem, a series of increasingly oblique sections was made until the longitudinal plane was reached. It was found that as the plane of section became more oblique, the secondary layers of the wall on one side of the sieve tube became brighter while those on the other side became darker in polarized light, until a certain plane of obliquity was reached. Presumably, when the plane of section was parallel to the longitudinal axis of the micelles on one side of the cell, it was cut transversely to those on the opposite wall. Under these conditions the wall on the former side showed the maximum brilliance, while the latter was dark in polarized light.

During the mature functioning stage, the sieve tubes contain many small spherical, discoid, or shell-shaped starch grains having the same size and appearance as those found earlier in the plastids. The sap, with its included starch grains, can be forced from end to end of the cell by slight pressure on the cover slip, and when a sieve tube element is cut and slightly pressed, the sap and starch grains flow out rapidly.

As early as late August, transitional stages to the winter condition of the mature sieve tubes may be found in white pine. These transition types may show some starch grains but also contain a number of small shredlike bodies (fig. 8). The thicker portions of these shreds stain red in Sudan III and IV. By the time that near or below freezing weather has come, a large proportion of the mature sieve tubes has passed into this shred-containing condition. The shreds are sometimes as much as 3 μ wide and 10 μ long, and are quite refractive. They are very numerous in portions of the sieve tube lumen, where they may be found collected into masses, oscillating at various rates, the smaller ones relatively rapidly.

The smaller granule type of plastid body of the sieve tube in its streaming stage and the shreds of the winter condition are preserved

by osmic acid. On the other hand, the shell-shaped starch grains of older plastids and of the mature sieve tubes are not found in sections which have been fixed in Flemming's solution and imbedded, sectioned, and stained by the usual techniques. Evidently the starch is hydrolyzed by these methods.

PARENCHYMA OF PHLOEM.—Concurrent with sieve tube differentiation, parenchyma of two general types develops in white pine phloem. These are ray cells (fig. 11) and phloem parenchyma formed from fusiform initials by septation (figs. 9, 10).

Ray initials give rise to two distinct types of xylem cells, ray parenchyma and ray tracheids, the latter lying horizontally along the margins of the rays. In the cambium the young ray parenchyma stain densely with anilin blue and appear dark in both radial (fig. 11) and tangential view. Soon, however, the erect cells of the phloem rays exhibit conspicuous pitting (figs. 15, 19, 20, 29) toward the adjacent sieve tubes. These are the "albuminous" cells of STRASBURGER (**21**); (see also **15**). They have been considered as analogous to the companion cells of angiosperms, because of their close association through pits, their dense protoplasmic content, and the fact that they die and are crushed along with the associated sieve tube elements. How much farther the analogy may be carried is doubtful, since these cells do not arise from a common fusiform initial with the sieve tube but from separate ray initials.

The remaining cells of the ray, most of which are horizontally elongated (fig. 11), soon vacuolate highly, become filled with starch or oil globules, and assume a relatively translucent appearance in tangential sections (figs. 10, 15, 19, 20, 21, 22, 24, 29). These cells persist after the death of the marginal cells and sieve tubes, and form, in the old cottony bark, a laterally arranged system of living cells providing conduction of nutrients to the phloem parenchyma and cork cambium. They increase in size, particularly by periclinal expansion, and remain alive until cut off by the phellogen. All the living ray cells have B vacuoles during the winter, and the persistent parenchyma cells just described contain numerous fat globules.

Phloem parenchyma formed by septation of fusiform initials is laid down relatively abundantly during the spring, forming annual

rings in the phloem by their radial alternation with bands of sieve tubes. Certain of these parenchyma cells may soon become filled with crystals and resin and die (fig. 10). Others vacuolate highly and store starch or oil in varying amounts (fig. 10, the clearer cells in the center). In many ways these cells compare with the horizontally elongated ray cells.

The great majority of the phloem parenchyma, however, rapidly develop large A vacuoles, become laden with tannin-like substances, and stain densely in fixed sections (figs. 9, 10, 16, 18). These cells form the layers of living tissue that persist in the older bark. Connected by living ray cells, these layers expand and crush the sieve tubes, gradually occupying the majority of the available space in the soft bark. They contain starch in addition to tannins during the autumn, and oil globules in the colder winter months. By renewed meristematic activity, certain of these cells give rise to successive cork cambia which produce the phellem of the outer bark.

DEATH OF SIEVE TUBE.—It is characteristic of the sieve tubes of all plants that, after a relatively brief functioning period—consisting of from a few days in the case of protophloem sieve tubes to a single season in most woody plants—the elements collapse, and death occurs. This is a necessary result of the nuclear disintegration and loss of semipermeability that initiate the functioning period of these elements. With continued reduction in the activity of the sieve tube cytoplasm, callus, which appears at the inception of this change, continues to develop. The callus rods of the sieve areas lengthen and enlarge, finally converging and forming the masses termed definitive callus (fig. 8). These masses are seen lining the sieve tube walls in figure 22, and in later stages may completely fill the lumina. Although this callus development may stretch the protoplasmic threads to many times their original length, there is no evidence that it "closes" the sieve "pores" in a physiological sense.

Definitive callus is simply a proliferation of the normal callus rods of the sieve areas, and occurs to some extent in all sieve tubes at the end of their functioning period. Callus may attain its ultimate development by the end of autumn in secondary phloem, and even earlier in primary phloem.

In white pine it persists in various stages of dissolution through-

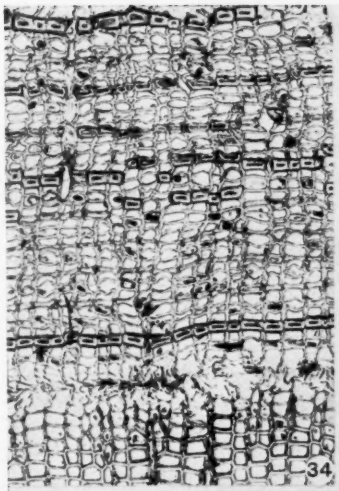
out the second season, and traces may be found in even older sieve tubes. The winter of the first year marks the end of the functional period of most of the sieve tubes however, for as the callus starts to disappear the parietal cytoplasmic layer dissolves. Air enters through the open lattices of the sieve areas of the dead sieve tubes, giving them a white glistening appearance. The walls become somewhat thinner as they dry. With drying, striations become more conspicuous in the secondary walls, emphasizing the characteristic helical structure.

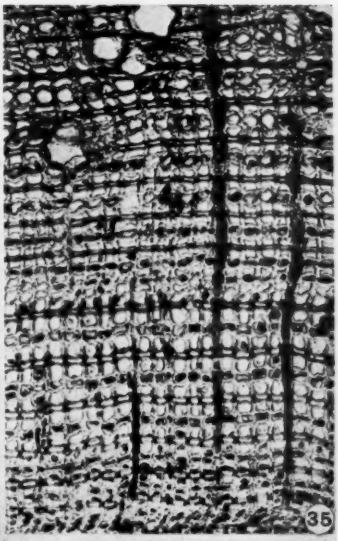
Starch-filled phloem parenchyma cells persist in the white cottony old phloem tissue. This remains alive for many years, to form the thick bark of the mature white pine tree.

Sieve tubes of other conifers

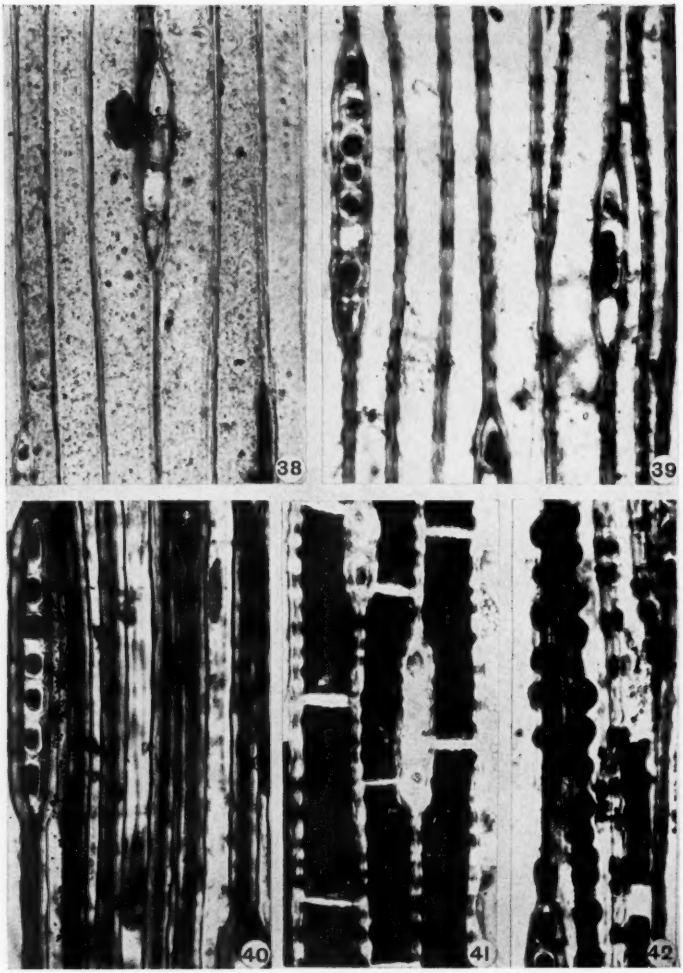
Figure 34 pictures the phloem of redwood, *Sequoia sempervirens*, and figure 35 that of incense cedar, *Libocedrus decurrens*. In contrast to white pine, these species have phloem fibers and show a stratified bark. The standard pattern of radial alternation of phloem elements in these species is fiber: sieve tube: phloem parenchyma: sieve tube: fiber, etc.; but examination of the figures shows some variation in pattern and considerable difference in the degree of wall thickening in different fiber layers. It is interesting to note that in the Cupressineae, Taxodineae, and Taxineae, secondary thickening is apparently restricted to the phloem fibers; whereas in Abietineae, where fibers are lacking, it is limited to sieve tube walls.

Figure 38 shows the very young phloem of redwood, a rumpled nucleus being present in the sieve tube slightly to the left of the ray in this picture. The walls are quite thin. Figures 39, 40, and 41 show the three tissues of functional phloem: sieve tubes (fig. 39), phloem fibers with their thick secondary walls (fig. 40), and phloem parenchyma (fig. 41). Again, phloem parenchyma cells are of two types, as are those of the rays. Albuminous cells are relatively fewer in number than in white pine. These cells are heavily pitted where in contact with sieve tubes. When the latter have definitive callus on their sieve areas, it occurs on the sieve tube side but is lacking on the ray cell side of their common pit pairs.





FIGS. 34–37.—Fig. 34, redwood phloem in transverse section with xylem at bottom and cambium somewhat crushed; ×200. Fig. 35, incense cedar phloem in transverse section with cambium at bottom and resin cysts near top; ×200. Fig. 36, definitive callus in senile phloem of fir; ×350. Fig. 37, slightly calloused sieve pits of same in longitudinal radial section; ×1000.



FIGS. 38–42.—Redwood. Fig. 38, very young phloem, sieve tube with crumpled nucleus. Fig. 39, mature phloem showing thickened primary walls with sieve pits. Fig. 40, phloem fibers in mature phloem showing secondary wall thickening. Fig. 41, phloem parenchyma with starch-bearing cells on extreme right and tanniniferous cells in remainder of section. Fig. 42, definitive callus in senile sieve tubes; all ×350.

Figures 42 and 44 show definitive callus in the sieve tubes of *Sequoia sempervirens*, and figures 36 and 37 show it in phloem cells of the root of *Abies concolor*,—figure 36 in longitudinal tangential view and figure 37 in longitudinal radial view.

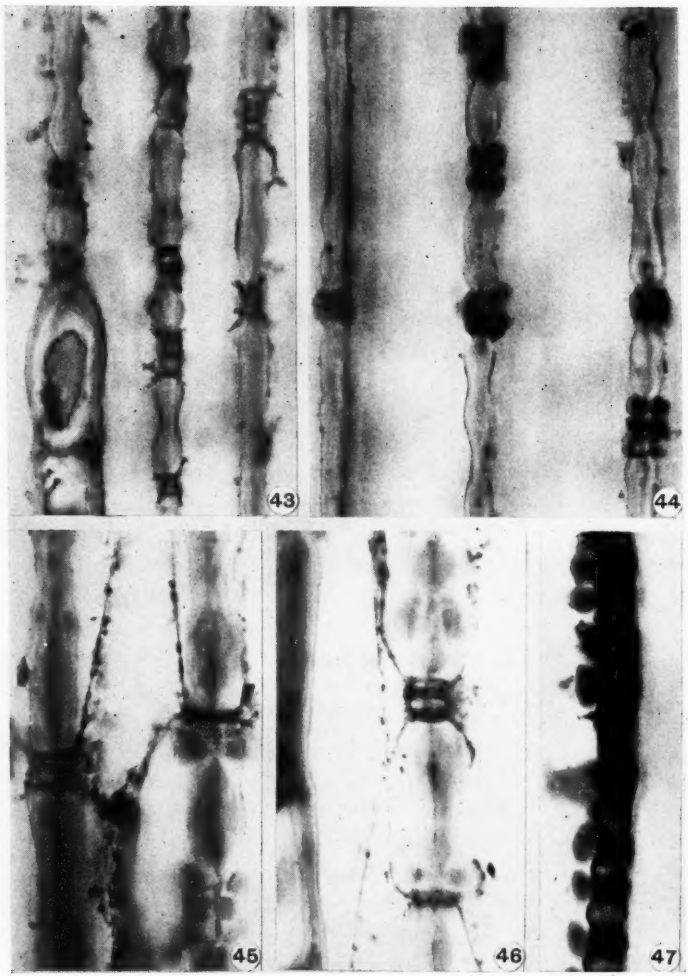
Threads in the sieve areas of *Sequoia sempervirens* are shown in figures 43, 45, and 46. They differ in this species from the other species studied in being extremely fine and forming a somewhat anastomosing network within the group involved in a single callus rod. This is particularly prominent in the connections of sieve tubes the protoplasm of which is in the fibroid, elastic condition just after disintegration of the nucleus. Figures 45 and 46 show the stringy nature of the parietal layer and internal strands during this phase of sieve tube ontogeny.

Figure 44 shows an early stage in definitive callus formation in *Sequoia sempervirens;* figure 47 a similar stage in *Abies concolor.*

The principal difference between the contents of the sieve tubes of the several conifers studied was found to be in the size and amount of the particles occurring in the elements during the winter. In *Taxus*, *Taxodium*, *Thuja*, and *Juniperus*, the large shreds noted in the Abietineae during the winter months were lacking, and fewer smaller bodies were observed in the sieve tubes. In some cases the sieve tubes were almost empty of visible contents except for large callus caps over the sieve areas. Callus formation was found to be essentially similar in all species studied. Sieve tube crushing was delayed in the Abietineae, as compared with the other species studied, probably owing to the secondary wall thickening. These elements persist, often for many years, in a semicollapsed condition, filled with air and devoid of protoplasm.

Microchemical tests on the walls of the various species studied show that the intercellular substance contains a high percentage of polyuronides; the primary walls are largely cellulose but contain some polyuronide; and the secondary walls of the sieve tubes of the Abietineae appear to be composed of relatively pure cellulose. While lignified fibers occur in many species, no lignin test could be obtained from any sieve tube walls.

In contrast to white pine, the writers were unable to find evidence of spiral structure in the sieve tube walls of *Juniperus virginiana*,



FIGS. 43–47.—Fig. 43, sieve tube walls of mature redwood phloem; ×1000. Fig. 44, early stage in definitive callus formation on sieve pits of redwood; ×1000. Fig. 45, cytoplasmic connections in sieve pits of redwood sieve tubes in swelled section; ×1400. Fig. 46, same showing details of anastomosing branched sieve connections; ×1400. Fig. 47, early stage in definitive callus formation in fr; ×1000.

either under polarized light or by the use of the swelling and staining agents.

When transverse sections of *Sequoia sempervirens* were treated in 30 per cent sodium hydroxide and subsequently stained in chlorozinc iodide, an inner wall layer was differentiated by its darker color, but it could not be proved to be secondary.

Discussion

Early literature on the phloem of conifers has been noted by HILL (**16**), and recent reviews (**19, 12, 18, 9**) cover the various aspects of phloem function. Most of the physiological interpretation of phloem structure relates to angiosperms (**12, 18, 9**). A brief review of the phloem anatomy of conifers might clarify some of the essential features of the mechanism that has been provided in these plants.

In contrast to the more specialized dicotyledons, the sieve tubes of conifers are very long and narrow. Cross walls of the type termed "sieve plates" are wanting, the sieve elements uniting longitudinally by diagonal common walls. Plasmodesmata between adjacent sieve elements are numerous but extremely tenuous. There are no companion cells, in the sense in which they occur in angiosperms.

On the other hand, sieve tubes in conifers resemble those in angiosperms in many ontogenetic features. They expand very rapidly, passing through a "bulging" stage; they lose their nuclei at maturity; their protoplasm goes through a similar series of changes, becoming fibroid, tough, elastic, and heavily staining; the mature elements often contain starch grains. Accompanying the denaturing process, the protoplast ceases streaming, loses its ability to accumulate solutes, and becomes completely permeable. In fact these protoplasmic changes, ending in complete permeability, seem to characterize sieve tubes in many plants (**10**), being the most constant feature of these elements that is known.

The enucleate permeable state of the mature functioning sieve tube in conifers predestines it to a short life, and, as in other vascular plants, these elements function for a season or less and then die, become air-filled and collapse or completely obliterate. This is shown graphically in figure 35. The sequence of cells in this section is sieve tube, parenchyma, sieve tube, fiber, etc. Starting at the cambium

along the lower edge, one may count twelve rows of differentiating cells to the third row of fibers, then twelve rows of mature elements, six of which are functioning sieve tubes. Above the sixth row of fibers, most of the sieve tubes are partially filled with definitive callus, denoting the end of their functioning life. Then there is a rapid change, the sieve tubes becoming smaller and flatter, the parenchyma cells larger and rounder, until between the twelfth and thirteenth fiber rows the latter cells are completely rounded and the sieve tubes badly crushed. This same course of ontogeny can be found in figure 34 and is characteristic of the phloem of all plants. It is not dependent upon cutting but may be found in stems killed *in toto*. It serves to emphasize the extremely narrow band of phloem tissue engaged in the longitudinal conduction of foods.

Rapid phloem exudation has recently been demonstrated in white pine (**10**). The technique necessary for the demonstration emphasizes again the nature of the mechanism and the limited band of tissue involved. In the experiments the hard bark and the soft white cottony phloem tissue is pared away, leaving exposed the thin, translucent, gelatinous layer of current season's phloem. When this is rapidly cut across, taking care not to go deep enough to open the xylem, several drops of a sweet sugary solution collect and flow from the wound. If any of the white cottony tissue is allowed to remain near the cut, the solution is rapidly absorbed into the old sieve tubes by capillarity, and the demonstration fails. Likewise if the xylem is cut, the solution is lost.

With the demonstration of the enucleate permeable condition of the sieve tubes of a wide variety of plants (**10**), there appears to be a rational basis upon which the structure of phloem in gymnosperms and angiosperms may be reconciled. Emphasizing the fundamental similarity in the mechanism of organic solute transport in all vascular plants, it points to a solution of this old problem by the demonstration of permeable conduits suitable to rapid longitudinal flow.

The tough fibroid nature of the parietal cytoplasmic layer in the enucleate sieve tube element has been described in cucurbits (**6, 9**), potato (**7**), and tobacco (**8, 13**). It is illustrated for white pine in figures 15, 25, 27, and 29, and for redwood in figures 45 and 46. In figure 15 the cytoplasm in the cell on the left of the ray was in a fluid

condition normal to living parenchyma cells. In the slightly more mature element on the right it was beginning to change, having shrunken and pulled away from the walls. In figures 25, 27, and 29, successive stages in the denaturing process are shown, the latter showing the heavy staining phase in which plasmodesmata are so prominent. Later, while remaining thin and fibroid, the parietal layer loses its affinity for stains and is demonstrated only by special methods. All attempts to find protoplasmic streaming in such protoplasm have failed, Brownian movement of the starch particles being the only motion observed.

CURTIS (**11, 12**), FISCHER (**14**), and others have objected that any method involving cutting the sieve tube system may fail to give a true picture of its contents. Therefore a branch of a five-year-old pine was shaved down, leaving a short thin continuous strip including the pith, wood, cambium, and bark. In such strips the inclusions of intact mature sieve tubes are in Brownian movement, and do not exhibit cyclosis.

Many phases of phloem structure still require investigation. The question of the sieve tube: companion cell relation is an open one. Although the origin of the erect ray cells in white pine is different from that of companion cells of angiosperms, the close association of these two cell types with enucleate sieve tubes is interesting. The common pitting and simultaneous death and crushing of marginal ray cells and sieve tubes indicate an essential interdependence between these elements. The protoplasm of the functioning sieve tube can scarcely be considered dead, and its activities may possibly be controlled by some adjacent nucleate cell. THOMPSON (**22**) was obviously in error in his description of companion cells in *Gnetum*. The elements he described as companion cells arise from cambial initials that give off xylem parenchyma on the inside, and not from division of the sieve tube mother cell after leaving the cambium. They do not have the thin common walls with sister sieve tube elements characteristic of companion cells. They are not crushed with the sieve tubes, but persist, and in some species differentiate into gelatinous fibers. They are undoubtedly parenchyma-like in nature throughout the period of sieve tube functioning.

Although it is often difficult to generalize from structure to func-

tion in plants, current theories on solute transport in the phloem differ so widely in the activity required of sieve tube protoplasm that studies of the type reported here should offer some concrete clues. It seems obvious that any hypothesis requiring rapid protoplasmic streaming, or a high activity state in the mature sieve tube protoplasm, is not supported by the cytological and anatomical data presented in this paper.

Summary

1. In Massachusetts, cambial division in white pine starts as early as February in some years, and is rapid during May. Phloem differentiation may lag until late summer or early fall, and daughter cells may remain in various stages of maturity through the winter.

2. Sieve tube differentiation starts with a localization of the nucleus in a protoplasmic bridge in the center of the cell. Rapid turgor expansion follows, bulging the pit areas and enlarging the cell. Protoplasmic streaming and vital stain accumulation indicate a high state of cell activity. As the sieve tube matures the nucleus disintegrates; the cytoplasm ceases streaming, becomes fibroid in consistency, develops an affinity for stains, and assumes a parietal position in the cell. The cell walls thicken, secondary walls are formed, and the connecting protoplasmic threads of the sieve areas stain heavily. Cessation of streaming, loss of the power to accumulate vital stains, and absence of plasmolysis in the presence of hypertonic solutions indicate a low activity state and a high permeability of mature sieve tube cytoplasm.

3. Denaturing of the protoplasm of the connecting threads is attended by callus formation in their immediate vicinity. Starch grains in the sieve tube plastids are released into the lumen and float free in the liquid contained there.

4. Senility of the sieve tube is attended by increase in the volume of callus on the sieve areas, a thinning down and loss of staining affinity by the cytoplasm, and a reduction in the numbers of starch grains.

5. Death of the sieve tube results in loss of the cytoplasm and callus, invasion of the lumen by air from the outer bark, and finally collapse of the elements.

6. White pine phloem contains no fibers; secondary wall thickening occurs in sieve tubes. Other genera of conifers have phloem fibers, and secondary wall thickening is confined to these elements, which are lignified.

7. Protoplasmic connections in the sieve areas of white pine are slender, solid, and fibroid in structure throughout the functioning period. Those of redwood are even more slender, consisting of anastomosing strands.

8. The picture of the functioning sieve tube of the conifers developed during these studies offers little support to theories of phloem transport requiring protoplasmic streaming or a high activity state in the mature sieve tube cytoplasm.

The investigations reported in this paper were initiated and conducted independently by the two writers. Since they dealt with the same plants from somewhat different points of view, it seemed advisable to combine the results in a single publication. Much of the cytological and histological work was carried out by Mrs. ABBE; the physiological investigations—particularly the plasmolysis and exudation experiments—were done by Dr. CRAFTS. The writers wish to express their appreciation to Professor I. W. BAILEY, of Harvard University, for assistance throughout the course of the work.

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HISTOLOGICAL STUDY OF THE DEVELOPING FRUIT OF THE SOUR CHERRY[1]

H. B. TUKEY AND J. ORAN YOUNG

(WITH EIGHT FIGURES)

Introduction

Earlier workers in the morphology of drupe fruits, as LAMPE (**11**), TSCHIERSKE (**19**), FARMER (**8**), and WINTON (**24**), dealt primarily with the structure of the mature fruit, and recorded but few significant structural changes in the developing fruit. For these studies, various species of *Rubus* were largely used, as *R. ideaus*, *R. fructicosus*, *R. strigosus*, and *R. occidentalis*, although LAMPE included a brief comparative discussion of the sour cherry, *Prunus acida*.

Later workers have been more concerned with the external or gross changes of the developing fruits, and have dealt principally with species of *Prunus*, as CONNORS (**5**), BLAKE (**2**), LILLELAND (**13**), HARROLD (**9**), DORSEY and MCMUNN (**6**), and TUKEY (**21**) with the peach (*Prunus persica* Stokes); LILLELAND (**12, 14**) with the apricot (*P. armeniaca* L.) and plum (*P. domestica* L.); and TUKEY (**20, 22**) with the sweet cherry (*P. avium* L.) and sour cherry (*P. cerasus* L.). These studies showed three characteristic growth periods in the developing fruit, designated (**22**) as: stage I, a period of rapid enlargement beginning about the time of full bloom; stage II, a mid-season period of retarded development varying in duration as correlated with the class of fruit and the season of fruit ripening; and stage III, a second period of rapid enlargement extending to fruit ripening (fig. 1).

Further study showed gross morphological changes of different parts of the fruit, such as the stony pericarp, nucellus and integuments, endosperm, and embryo. These were correlated with the three major growth stages of the fruit by TUKEY (**20, 21, 22**), LILLELAND (**14**), and HARROLD (**9**). LOTT (**15**), and ADDOMS, NIGHTINGALE, and BLAKE (**1**) showed chemical changes in the

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fleshy and stony pericarp, and LOTT (**15**) and TUKEY and LEE (**23**) in the seed and embryo as related to these stages.

The histological and structural changes have been less well investigated. The present study is an attempt to show these changes in the developing fruit of the sour cherry (*Prunus cerasus* L. var. Montmorency), from the closed bud (18 days before full bloom) to the ripe fruit. It includes a treatment of the principal tissues of the pericarp during each of the three growth stages and during the pre-bloom stage.

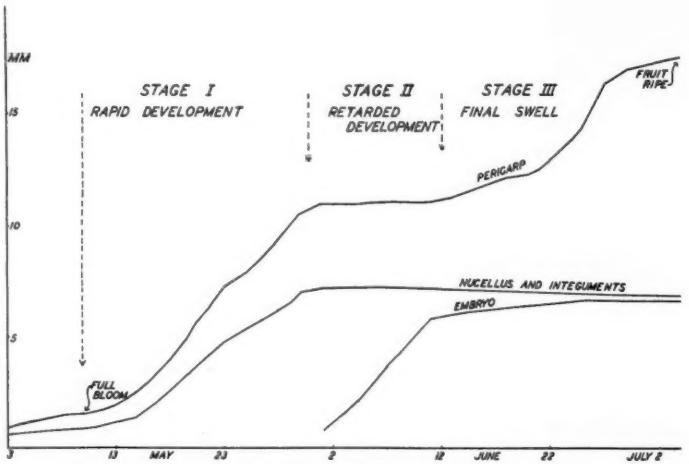


FIG. 1.—Growth stages in development of fruit of sour cherry from pre-bloom to fruit ripening. After TUKEY (**22**).

Material and methods

Montmorency, a mid-season variety, ripens 57 to 59 days after full bloom. In this variety stage I is of 20 to 22 days duration; stage II, 12 to 16 days; and stage III, 21 to 23 days (**22**).

MATERIAL.—Fresh material from the season of 1938 and material fixed in three different ways and during four different seasons were used, as follows:

1. Season of 1930, ripe fruit in 95 per cent alcohol.
2. Season of 1931, at 2 to 3 day intervals from April 20 to May 26, in Karpechenko's chromacetic and in formalin-acetic alcohol.

3. Season of 1932, daily from June 4 to June 26, in formalin-acetic alcohol.
4. Season of 1938, at 2 to 5 day intervals from May 24 to July 19, in formalin-acetic alcohol.

Although there was some variation in the number of days from full bloom to fruit ripening in the different years, it was possible to correlate the material. A growth curve was made for each season based on gross measurements of the fruit and examination of the development of the embryo and stony pericarp (fig. 1). The dates of full bloom and of fruit ripening being known, it was possible to locate definitely the beginning, middle, and end of each of the three stages of fruit development for the material from all four seasons, and to place one curve upon another. Samples from the various seasons were accordingly selected for study at these points and were found to fit together almost perfectly to form a four season record of development.

FIXATION, SECTIONING, AND STAINING.—Material used in the study of the pre-bloom stage and parts of stage I was killed and fixed in Karpechenko's chromacetic and run up into paraffin; then sectioned with a rotary microtome and stained with safranin, orange G, and crystal violet.

Larger specimens used in the study of stages I, II, and III to fruit ripening were either examined fresh or killed and fixed in a mixture of 100 cc. of 50 per cent alcohol, 23 cc. of glacial acetic acid, and 67 cc. of formalin, and held in this mixture until sectioned either freehand or on a sliding microtome. All freehand sections of this type were stained in safranin and light green. Most of them were mounted in balsam, but some were examined immediately in liquid mounts and found satisfactory for outline drawings.

When the stony pericarp had begun to harden (stage II), there was some difficulty in getting good sections which contained both stony and fleshy pericarp. Attempts at freezing and holding the fruit in various pin devices so as to exert no pressure on the fleshy pericarp were not superior to making numerous freehand sections and selecting the better ones for study. Sections made from fresh material and from that in formalin-acetic alcohol were equally satisfactory.

When the stony pericarp had become too hard to secure complete

sections through the entire pericarp, freehand sections for detailed study were made separately in three regions: the fleshy pericarp, portions of the adjacent stony and fleshy pericarps, and the stony pericarp.

For complete sections of the stony pericarp from ripe fruit, the pit was removed and ground successively on coarse sandpaper, on fine sandpaper, on a medium carborundum stone, and on a fine oil stone. When the section was 2 mm. thick, it was mounted on a glass slide with Duco household cement and ground further to the desired thickness. This cement hardened rapidly, held the mount firmly, and permitted staining without separation from the slide.

The epidermis was examined in surface view by stripping from the fruit. No stain was needed since the cells were sufficiently pigmented to show good contrast.

MEASUREMENTS AND DRAWINGS.—All comparative outline drawings were made from cross sections through the center of the fruit perpendicular to the polar axis. Comparative measurements and drawings of number and size of cells were made from sectors at the cheek of the fruit, as this has been shown (**22**) to be the point at which development is most regular. The cheek diameter may be defined as a line passing through the center of the fruit perpendicular to both the longitudinal axis and the suture diameter.

Outline drawings where no cell details were desired were made with the use of a Bausch & Lomb triple purpose microprojector using a 16 mm. objective and a constant set-up. Detailed drawings were made with a camera lucida, always to the same scale, and with the same set-up of equipment to give identical enlargement.

Counts of numbers of cells and measurements of cell size were made with the use of both fixed and sliding ocular micrometers, and checked against camera lucida drawings. For cell size, an average was taken of several cells which seemed typical of the tissues under consideration. Mitotic figures were noted and recorded as evidence of cell division.

Investigation

GENERAL CONSIDERATIONS OF TISSUES INVOLVED

Three principal tissues compose the ovary wall of the cherry: inner and outer epidermis, stony pericarp, and fleshy pericarp. The

gross development of the fruit and of these tissues is shown in the accompanying series of outline drawings (fig. 2), beginning 18 days before full bloom and continuing to fruit ripening. The development at the beginning, middle, and end of each stage is shown.

The stony pericarp may be divided into an inner and an outer layer, and the fleshy pericarp into an innermost layer of small thin-walled parenchyma, a middle region of large thin-walled parenchyma, and an outer or hypodermal layer of collenchyma. The hypodermal layer and the epidermis together constitute the "skin" of the fruit. Not all of these tissues are sharply defined in the earlier stages of development, and they are outlined in the accompanying drawings only when they are to be easily seen at that stage.

In subsequent detailed drawings (figs. 3, 4, 5) of the cellular changes which these tissues undergo, the same relative positions on the page are retained for drawings of similar stages as those of gross development in figure 2, to facilitate comparison. In tables 1 and 2 the counts of cell number and measurements of cell size in the tissues involved are for corresponding stages of development. In the discussion which follows, the tissues are taken up in the following order: stony pericarp, fleshy pericarp, and outer epidermis.

Stony pericarp

Pre-bloom.—Eighteen days before full bloom the innermost portion of the ovary wall, which becomes the stony pericarp at fruit ripening, is composed of the single row of cells of the inner epidermis and an additional three or four layers of cells of the pericarp (fig. 3*A*). The cells of the inner epidermis are block-shaped while the others are elongate in the direction of the central axis of the fruit.

The line of demarcation between that portion of the pericarp which is destined to become stony and that which is to remain fleshy is discernible even at the beginning of the period, the cells of the former being smaller, in general, than those of the latter. It is not possible at this date to separate a single bordering cell of the one from the other. The relatively smaller size of cells of the stony pericarp persists to maturity.

Soon after the beginning of this period the cells of the inner epidermis elongate tangentially, as seen in cross section, and just before full bloom they divide in a transverse direction (fig. 3*B*, *C*).

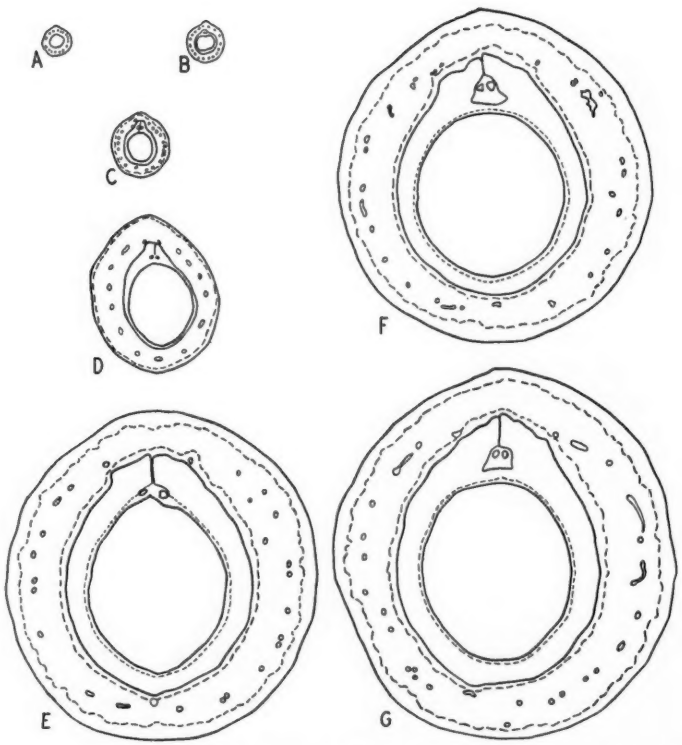
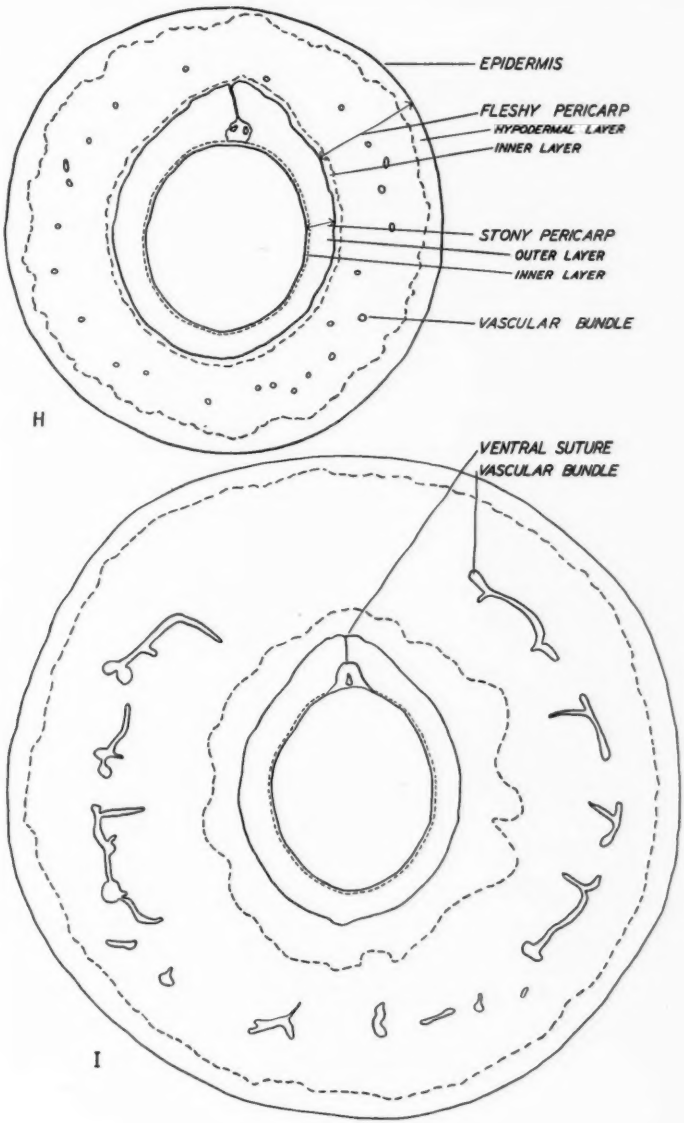


FIG. 2.—Development of cherry fruit as seen in transverse section, from 18 days before full bloom to fruit ripening. *A:* 18 days before full bloom; *B:* 6 days before full bloom; *C:* full bloom; *D:* 11 days after full bloom; *E:* 20 days after full bloom; *F:* 26 days after full bloom; *G:* 36 days after full bloom; *H:* 40 days after full bloom; *I:* fruit ripening. 57 days after full bloom.



(FIG. 2.—*Continued*)

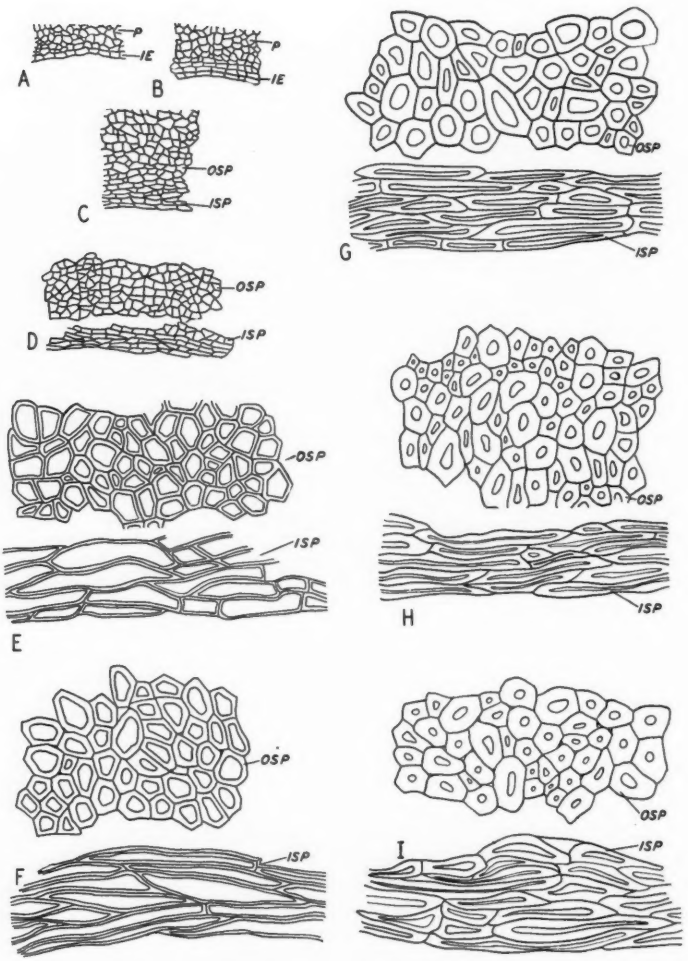


FIG. 3.—Development of stony pericarp (camera lucida drawings of transverse sections ×163). *p*, pericarp; *ie*, inner epidermis; *osp*, outer stony pericarp; *isp*, inner stony pericarp. *A:* 18 days before full bloom; *B:* 6 days before full bloom; *C:* full bloom; *D:* 11 days after full bloom; *E:* 20 days after full bloom; *F:* 26 days after full bloom; *G:* 36 days after full bloom; *H:* 40 days after full bloom; *I:* fruit ripening, 57 days after full bloom.

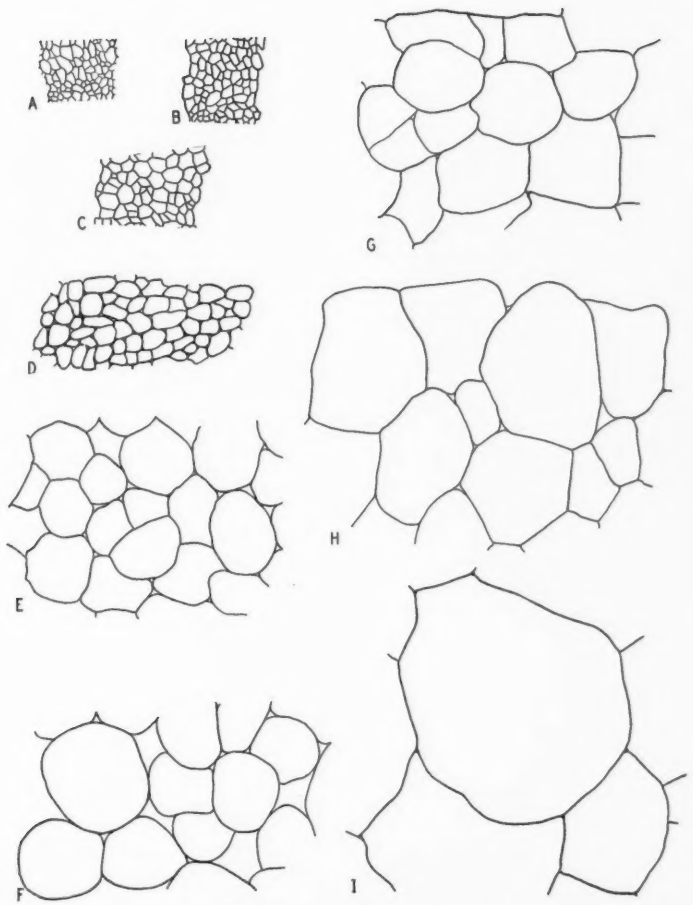


FIG. 4.—Development of fleshy pericarp, in region between hypodermal layer and ring of vascular bundles (camera lucida drawings of transverse sections ×163). *A:* 18 days before full bloom; *B:* 6 days before full bloom; *C:* full bloom; *D:* 11 days after full bloom; *E:* 20 days after full bloom; *F:* 26 days after full bloom; *G:* 36 days after full bloom; *H:* 40 days after full bloom; *I:* fruit ripening, 57 days after full bloom.

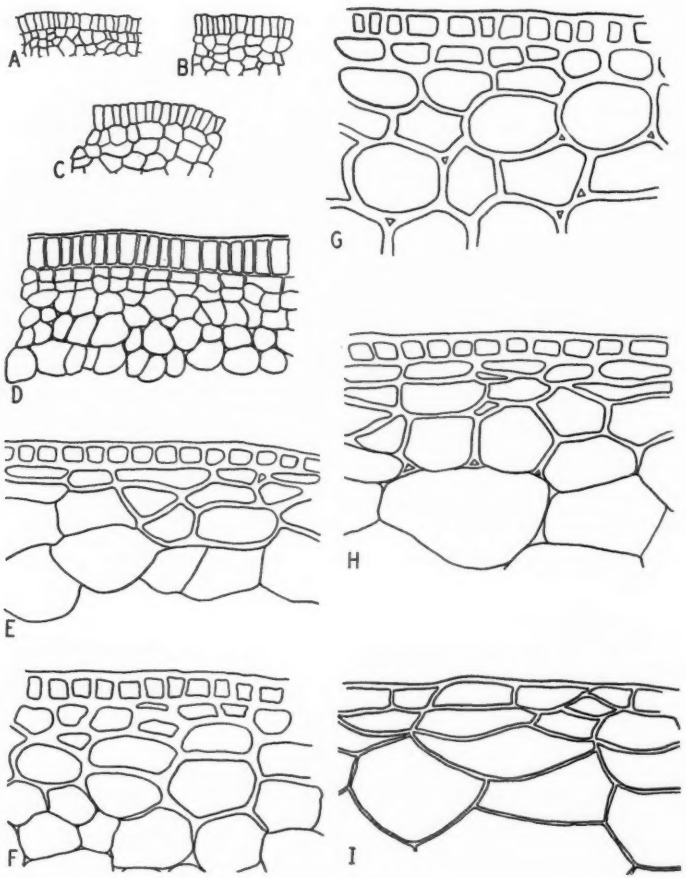


FIG. 5.—Development of epidermis and hypodermal layer (camera lucida drawings of transverse sections ×163). *A:* 18 days before full bloom; *B:* 6 days before full bloom; *C:* full bloom; *D:* 11 days after full bloom; *E:* 20 days after full bloom; *F:* 26 days after full bloom; *G:* 36 days after full bloom; *H:* 40 days after full bloom; *I:* fruit ripening, 57 days after full bloom.

TABLE 1

CHANGES IN NUMBER OF LAYERS OF CELLS IN A RADIAL DIRECTION THROUGH CHEEK OF CHERRY FRUIT FROM 18 DAYS BEFORE BLOOM TO FRUIT RIPENING

Stage of development	Days before or after full bloom	Fleshy pericarp				Stony pericarp		
		Epidermis and hypodermis	Large-celled parenchyma	Small-celled parenchyma	Total	Outer	Inner	Total
Pre-bloom	−18	4–5	11	.....	15–16	4–5	.....	4–5
Pre-bloom	− 6	4–5	11	.....	15–16	7–8	.....	7–8
Full bloom	0	5	17	.....	22	10	4	14
Middle stage I	+11	5–6	19–21	.....	24–27	12	6–7	18–19
End of stage I	+20	5–6	18–19	3–4	26–29	17–18	6–7	23–25
Middle stage II	+26	5–6	19	3–4	27–29	20	6–7	26–27
End of stage II	+36	5–6	19	3–4	27–29	20	6–7	26–27
Middle stage III	+40	5–6	19	3–4	27–29	20	6–7	26–27
End of stage III	+57	5–6	19	3–4	27–29	20	6–7	26–27

TABLE 2

CHANGES IN SIZE OF CELLS IN A RADIAL DIRECTION THROUGH CHEEK OF CHERRY FRUIT FROM 18 DAYS BEFORE FULL BLOOM TO FRUIT RIPENING

Stage of development	Days before or after full bloom	Epidermis		Fleshy pericarp		Stony pericarp
				Parenchyma		
		Radial diameter (μ)	Tangential diameter (μ)	Radial diameter (μ)	Tangential diameter (μ)	Cross section (μ)
Pre-bloom	−18	17	10	18	13	10
Pre-bloom	− 6	18	10	18	16	10
Full bloom	0	22	11	20	20	11
Middle stage I	+11	28	13	32	32	12
End of stage I	+20	35	22	83	83	28
Middle stage II	+26	35	28	90	90	30
End of stage II	+36	35	31	92	92	30
Middle stage III	+40	35	±39	±180	±100	30
End of stage III	+57	35	±68	350–500	100–250	30

Together with a few adjacent cells of the pericarp which are similar in shape and orientation, they form a band or "hoop" of transversely elongated cells bounding the inner ovary wall. Since they lie at right angles to the other cells of the stony pericarp, the boundary between the two is sharp and makes a clear division between the sections which at maturity are designated inner stony pericarp and outer stony pericarp.

TSCHIERSKE (**19**) pictures a similar condition in the pericarp of *Potentilla anserina;* and LAMPE (**11**), WINTON (**24**), and FARMER (**8**) observed an identical situation in species of *Rubus*. FARMER records "a chain of such fibres running around the fruit cavity resembles a segmented ring or hoop . . . . and the whole of the interior of the ovary appears to be bounded by these 'rings' which run in a transverse direction round it. . . . . the rest consists of fibres. . . . . whose longest axis runs in a direction at right angles to that of the cells just described. The result of this arrangement is that the ovarian cavity is protected by a band of tissue, of which one half is fitted to stand strains and stresses in one direction, while the other half is equally well capable of resisting similar strains and stresses in a direction at right angles with the first" (**8**).

By rapid division within the tissue which is to become the stony pericarp, the number of cells is nearly trebled by the time of full bloom. The diameter increase of cells is, however, only 10 per cent. By the time of full bloom the tissue is well defined as a region of small compact cells scarcely half the diameter of the adjacent cells of the fleshy pericarp. The inner stony pericarp is approximately four cells thick and the outer stony pericarp ten cells thick.

It may be well to emphasize that the groups of cells which give rise to the stony pericarp apparently do not give rise to cells of the fleshy pericarp, or vice versa. It is not a case of the entire pericarp being derived from a common group of cells, some of which toward the center of the fruit become sclerenchymatous and some of which toward the periphery remain parenchymatous and enlarge. Nor is it a case of progressive hardening of cells of a common tissue from the center of the fruit outward in a radial direction and the enlargement of the remaining unhardened cells to comprise the fleshy pericarp. On the contrary, the two tissues appear to be derived each from dis-

tinct groups of cells which are early separated by characteristic size, shape, and frequency and periodicity of division. There is no transition in size from the smaller cells of the tissue of the stony pericarp to the larger cells of the fleshy pericarp. Moreover, mitotic figures are relatively more abundant in the tissue of the stony pericarp at the time that greatest increase in number of cells by actual count occurs, and a similar condition obtains in the tissue of the fleshy pericarp.

STAGE I.—During stage I (fig. 3*C*, *D*, *E*) the total thickness of the wall of the stony pericarp at the cheek increases approximately five times. This is brought about by a large increase in both number and size of cells. Enlargement of cells for both the inner and outer sections is marked,—an increase of approximately sixteen times in length and two or three times in width. In the inner layer, elongation takes place in a tangential direction, as seen in cross section, but in the outer layer elongation is in the direction of the central axis of the fruit. Scattered groups of cells may be found which are oriented in planes at variance with the general pattern.

By the end of the period the number of cells along the radius of the pit at the cheek has increased from fourteen to twenty-four, the greatest increase occurring in the outer stony pericarp. Active cell division continues later than in the fleshy pericarp. In the inner stony pericarp no cell divisions have been observed after the middle of stage I, but in the outer stony pericarp the greater increase occurs in the latter half. Likewise the increase in the size of all cells takes place largely in the latter half of the period (fig. 3*E*). By the middle of stage I the cells have increased from 10 to 12 μ in transverse diameter, while at the end of stage I they measure 28 μ.

Cell walls become progressively thicker but do not harden appreciably until near the end of the period. At the end of the stage the pit is too hard to section without special treatment, although the walls have not yet attained more than one-fifth the thickness found in the ripe fruit.

STAGE II.—Thickening and hardening of the cell walls of the stony pericarp are the most characteristic developments of stage II (fig. 3*E*, *F*, *G*). Although maturation of the tissue is progressing rapidly as evidenced by thickening and hardening of the cell walls, yet ac-

tive cell division occurs here as late as, if not later than, in any other part of the pericarp. This may be explained by the fact that hardening does not occur uniformly throughout the stony pericarp. It begins near the apical end, at the hilum, and extends downward along the ventral sutures toward the chalaza, before finally including the entire stony pericarp. These observations agree fairly completely with those of MIKI (16) and RAGLAND (17) for the peach, and BRADBURY (4) for the sour cherry. At the completion of the period the stony pericarp is composed of twenty-six to twenty-seven layers of very thick-walled, lignified cells. This is nearly half the total number of layers in the entire pericarp, but because of the much smaller size of the cells of the stony pericarp, they represent much less than half the thickness of the ovary wall.

No cell divisions have been observed in this region beyond the middle of stage II. The cells are living, however. Nuclei are abundant, and by their bright reaction to safranin suggest activity. Such suggested activity would seem in agreement with the tremendous thickening of the walls and their impregnation with materials. The lumen is frequently scarcely larger than the nucleus.

STAGE III.—During stage III (fig. 3*G*, *H*, *I*) there is slight increase in hardness and brittleness of the tissue, associated with loss of moisture (15). Although by the time of fruit ripening most of the cells have become matured as sclerenchyma, it is still possible to find occasional living cells with active nuclei.

FLESHY PERICARP

PRE-BLOOM.—Eighteen days before full bloom the fleshy pericarp is composed of nearly isodiametric, parenchymatous cells (fig. 4*A*, *B*). There is a suggestion of tangential elongation near the epidermis and a gradual change in orientation to a slight radial elongation near the stony pericarp. Increase in the thickness of the tissue, which is a little more than doubled, is due mostly to cell division, although there is some very slight cell enlargement. In a radial direction through the cheek, from the epidermis to the stony pericarp, the cells increase approximately 40 per cent in number during the period.

STAGE I.—During stage I the number of cells across the cheek from epidermis to stony pericarp is increased 20–30 per cent, most

of the increase occurring during the first half of the period. No mitotic figures could be found after the tenth day from full bloom. ADDOMS, NIGHTINGALE, and BLAKE (1) and RAGLAND (17) have noted a similar rapid cell division in the peach following full bloom, and its cessation early in stage I; and TETLEY (18) has found that growth in the apple after the time of fruit set is mostly a matter of cell enlargement.

Cell diameter is quadrupled, from 20 to 83 μ, the increase occurring mostly in the latter half of the period. Intercellular spaces become numerous and prominent toward the end of the period.

A hypodermal layer five or six cells thick is differentiated just beneath the outer epidermis during this period, by considerable thickening of the cell walls (fig. 5*D*, *E*). The cells attain but little greater size than those of the epidermis, but become somewhat elongated tangentially as seen in cross section. The shape of the cells of the thin-walled parenchyma remains essentially the same throughout the period, except in the outer portion where some tangential elongation takes place (fig. 4*C*, *D*, *E*).

A layer of small isodiametric cells three or four cells wide is formed adjacent to the stony pericarp during the latter part of the period. With the possible exception of this layer, there is no further cell division in the fleshy pericarp after completion of stage I. The combined activity of cell division and enlargement throughout the fleshy pericarp results in an increase in thickness of seven times. Nearly the same figure applies to the stony pericarp; hence the growth is fairly uniform for both tissues. Cell division occurs later in the stony than in the fleshy pericarp, notwithstanding the earlier differentiation of the former (fig. 4*E*, *F*, *G*).

STAGE II.—During stage II there is some small enlargement of the cells of the fleshy pericarp, from a diameter of 83 to 92 μ. Tangential elongation of the cells just beneath the outer epidermis continues slowly. Intercellular spaces remain prominent. There may be some cell division in the thin layer of small cells adjacent to the stony pericarp, but otherwise there is none. Increase in thickness of the fleshy pericarp is not more than 6 per cent.

STAGE III.—During stage III, the final swell (fig. 4*G*, *H*, *I*), four divisions of tissue may be recognized, exclusive of the outer epider-

mis: (a) the hypodermal layer of collenchyma; (b) a peripheral layer of thin-walled parenchyma extending from the hypodermal layer to a line just inside the ring of vascular bundles; (c) a layer of radially elongated cells extending from this line nearly to the pit; and (d) a thin layer of small cells adjacent to the pit.

While for convenience an arbitrary classification of tissues has been made, it must be realized that there is a gradual transition from a tissue in which one type of cells predominates to that in which another is dominant. As seen in some sections, thin-walled cells of the fleshy pericarp may occasionally come in direct contact with the epidermis; or the layers of tangentially stretched and flattened collenchyma of the hypodermal layer may be wanting. Likewise the collenchyma gives way gradually to the three or four layers of large, more nearly roundish cells of thin-walled parenchyma. These in turn give way to radially elongated but notably widened or obovate cells, which pass to progressively more narrow, radially elongated cells toward the pit.

The chief feature of this stage, often called the final swell, is the increase in size of the individual cells of the fleshy pericarp, the largest indicating an increase of as much as twenty-five times in diameter from the size at full bloom. The cells of the hypodermal layer become enlarged in a tangential direction, similar to the adjacent cells of the outer epidermis. Those in the outer portion of the fleshy pericarp increase several times in diameter and become roundish oval in shape, with the greatest diameter parallel to the periphery of the fruit. They become progressively larger from the hypodermal layer inward to the region of the vascular bundles, and the tangential elongation becomes progressively less marked. Near the ring of vascular bundles the cells are essentially round.

In the middle region of the fleshy pericarp tremendous radial elongation occurs, an increase of four to six times, and some enlargement tangentially. The tangential enlargement is progressively less toward the stony pericarp. As would be expected from the rapid stretching, there is a decrease in the thickness of cell walls. Intercellular spaces, which were so frequent and conspicuous during stage II, are now difficult to find. This may be due in part to the relatively greater size of the individual cells and the attendant decreased like-

lihood for the inclusion of intercellular spaces in a cross section. Or it may be that increase in pressure within the cells accompanying enlargement causes the relatively thin walls to lie more closely together. ADDOMS, NIGHTINGALE, and BLAKE (1) found intercellular spaces much less conspicuous in the peach than in the apple, and suggested that in the case of the peach, larger areas of the cell walls are in contact with the walls of adjacent cells than in the apple. TSCHIERSKE (19) observed the absence of intercellular spaces in the region of elongated cells in the fleshy pericarp of the red raspberry. At all events the nature of the tissue is one of closely compressed and compacted cells with inconspicuous intercellular spaces, a striking contrast to the description which may be found in the literature of loose-fitting cells with large intercellular spaces.

The band of three to five rows of very small isodiametric parenchyma cells which lie adjacent to the pit makes no notable increase in size during this period. The cells lie between the large radially elongated cells on the one side and the cells of the stony pericarp on the other. Although the line of demarcation between the stony and the fleshy pericarp is distinct, cells of the one are more or less dovetailed between cells of the other, so that the outer surface of the stony pericarp when separated from the fleshy pericarp is slightly rough and somewhat pitted.

The total number of rows or layers of cells in a radial direction through the cheek of the fleshy pericarp has been counted as twenty-seven to twenty-nine (table 1). It is of interest to note that LAMPE (11) gives twenty-eight as the number from observations of *Prunus acida* made in 1886. Further, the number of layers of cells is nearly equally divided between the stony pericarp and fleshy pericarp, being twenty-six to twenty-seven for the former—a total of fifty-three to fifty-six.

VASCULAR BUNDLES

The ventral and dorsal carpillary bundles lie wholly without the stony pericarp, although close to it. Two bundles which supply the two ovules, called in the peach "funicular bundles" by RAGLAND (17), lie at either side of the ventral suture and adjacent to the ovarian cavity (fig. 2). There are no "pit bundles" traversing the stony pericarp in the cherry, such as he describes for the peach.

Extending through the fleshy pericarp in a direction parallel with the central axis, as seen in cross section, is a ring of vascular bundles, generally eighteen to twenty in number. They are differentiated as early as 18 days before full bloom, but the walls of the xylem elements do not become sufficiently thickened to become conspicuous until about the time of full bloom. They correspond to the ring of "pit bundles" in the stony pericarp of the peach (**18**).

As the fruit develops, the number of bundles in the ring is not increased. The proportion of vascular tissue to the total area of the pericarp as seen in cross section thus appears much greater in the early stages than in later ones, as shown in outline drawings (fig. 2). The vascular system, moreover, is extended progressively throughout the tissue as the fruit develops, largely by transverse divergence of smaller bundles from the main bundles, similar to the condition reported in the peach by RAGLAND. At maturity the vascular bundles ramify throughout the fleshy pericarp to give a skeleton network of conductive tissue.

Throughout the development of the fruit until just before ripening there are certain cells which are conspicuous because of the granular nature of their contents and their absorption of safranin and orange G stains. They tend to form a loose network throughout the fleshy pericarp, with greatest concentration around the vascular bundles and in a layer several cells thick just beneath the outer epidermis. They are slightly larger than the average for cells of the fleshy pericarp. In the ripe fruit the granular nature of the contents disappears and the cells become indistinguishable from the adjacent parenchyma.

EPIDERMIS

The outer epidermis is a single row of cells covered externally by a cuticle which is continuous except where interrupted by stomata. The cells of the epidermis are well differentiated in material collected 18 days before full bloom (fig. 5*A*). At this time they are elongated radially, being about twice as long as broad. The development is similar to that in the peach as described by DORSEY and POTTER (**7**), excepting that there are no hairs, of course. During the entire period they increase slowly in size, chiefly in a radial direction

(fig. 5*B*). Eighteen days before full bloom an average of 312 cells was counted in the periphery of a cross section through the cheek. Two days before full bloom this number had increased to 390 cells.

During stage I (fig. 5*C*, *D*, *E*) the epidermal cells increase decidedly in both number and size. At 1 day after full bloom, 550 cells were counted in the circumference of a cross section. At the end of stage I this number had reached slightly more than double, 1187. The increase in numbers takes place very rapidly in the first 10 days of the stage; no divisions were observed in the latter part. Cell enlargement in both radial and tangential diameters occurs mostly toward the end of the period. The cuticle attains full thickness during the latter part of the period, and the radial walls are considerably thickened.

During stage II (fig. 5*E*, *F*, *G*) development of the epidermis consists in a slight tangential elongation of the cells and additional thickening of the walls. No cell divisions occur.

During stage III (fig. 5*G*, *H*, *I*) there is great enlargement of the epidermal cells in tangential directions, accompanying the large increase in the surface area of the fruit. Since cell division ceases during stage I, any increase in the size of the fruit must be compensated for by a tangential stretching of the epidermal cells. Actually the tangential diameter is more than doubled during this period, while there is a slight decrease in the radial diameter as well as in the thickness of the cuticle. The drawings (fig. 5*I*) are similar to those by Dorsey and Potter (7) for the peach, and Kosemanoff (10) for the sweet cherry.

Stomata.—The stomata are unevenly distributed over the surface of the cherry. They are numerous near the apex and few near the stem end. By 18 days before full bloom they are fully differentiated (fig. 6*A*).

Since the epidermal cells increase materially in number during later stages and no more stomata are formed, they appear relatively numerous and close together in this early stage. The guard cells increase in size as the fruit develops (fig. 6*B*, *C*) but the increase is much less than that of typical epidermal cells. At 18 days before full bloom the guard cells measure 13 μ in length; at full bloom, 23 μ; during the middle of stage I, 25 μ; at the end of stage I, 31 μ; during

the middle of stage II and at the end of stage II, 34 μ; at the end of stage III, 47–49 μ.

During all stages from the middle of stage I to fruit ripening, the walls of the cells adjacent to the guard cells are very much thinner than those of typical epidermal cells. In the ripe fruit there is no development of chromoplasts in the guard cells nor in the cells immediately adjacent. In fresh unstained material the cell contents appear yellowish or straw-colored, in contrast to the bright red color of epidermal cells well supplied with chromoplasts.

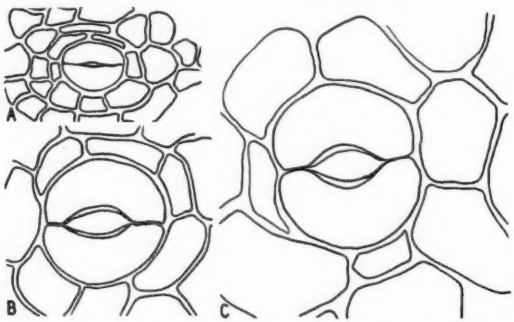


FIG. 6.—Development of stomata, guard cells, and adjacent epidermal cells in surface view (camera lucida ×480). *A:* 18 days before full bloom; *B:* 20 days after full bloom; *C:* fruit ripening, 57 days after full bloom.

The general appearance resulting from these areas of contrasting colors is of dots which may be easily seen with the naked eye, and suggests lenticels. DORSEY and POTTER (7) described a similar appearance in the peach. No true lenticels have been observed in the material studied. Occasional rupture of the epidermis from mechanical causes has been seen; and discolored areas were found, the result of spray materials or some mechanical injury. Even in ripe fruit which had been allowed to remain on the trees until September 20, 60 days after commercial harvest, stomata and guard cells were still intact (fig. 6*C*) and no lenticels were found.

Discussion

The shape of the cells of the fleshy pericarp from the periphery of the fruit to the stony pericarp, as seen in cross section, appears

much as it might if each cell was subjected to relatively equal internal force of expansion in all directions, such as osmotic pressure.

The cells nearest the pit can expand very little except in a radial direction, because of the close position tangentially of other cells exerting similar pressures toward them. The cells surrounding the first ones, besides making a radial elongation, can now expand slightly in a tangential direction, since the diameter of the fruit has become greater and the distance in a tangential direction between the theoretical centers of the rows of cells has become greater, similar to the increase in distance between the spokes of a wheel the farther from the hub. Progressively toward the periphery of the fruit the tangential pressure from adjacent cells becomes less, so that the cells assume an obovate shape, with the small end pointing toward the center. Finally, near the periphery of the fruit the internal cell pressure is equally balanced by the inward pressure of the stretching epidermis and hypodermal layer, by the outward pressure of inner cells, and by the tangential pressure of adjacent cells, so that they assume a roundish shape. In some instances these cells may even be broadly oval. The cells of the hypodermal layer and epidermis, being subjected to outward pressure from the expanding thin-walled parenchyma, become stretched tangentially and flattened.

In figure 7 is shown a series of camera lucida drawings of the cells in a radial sector of the ripe fruit from the stony pericarp to the outer epidermis, illustrating how closely the cell shapes and sizes conform to the preceding description. ADDOMS, NIGHTINGALE, and BLAKE (1) have remarked upon the radial elongation of cells of the fleshy pericarp of the peach near the pit and the more ovoid shape further out.

The gross size changes in the fruit during the three stages of development are seen from this study to be made up of cell divisions and cell enlargements in varying proportions in different tissues at different times. Figure 8 shows the amount of increase in a transverse direction through the wall of the fruit at the cheek. It has been prepared by calculation from the actual numbers and sizes of cells in the different tissues at the beginning, middle, and end of each growth stage (tables 1 and 2). While the general nature of the curve is similar to that for the total diameter of the fruit in figure 1, it differs in

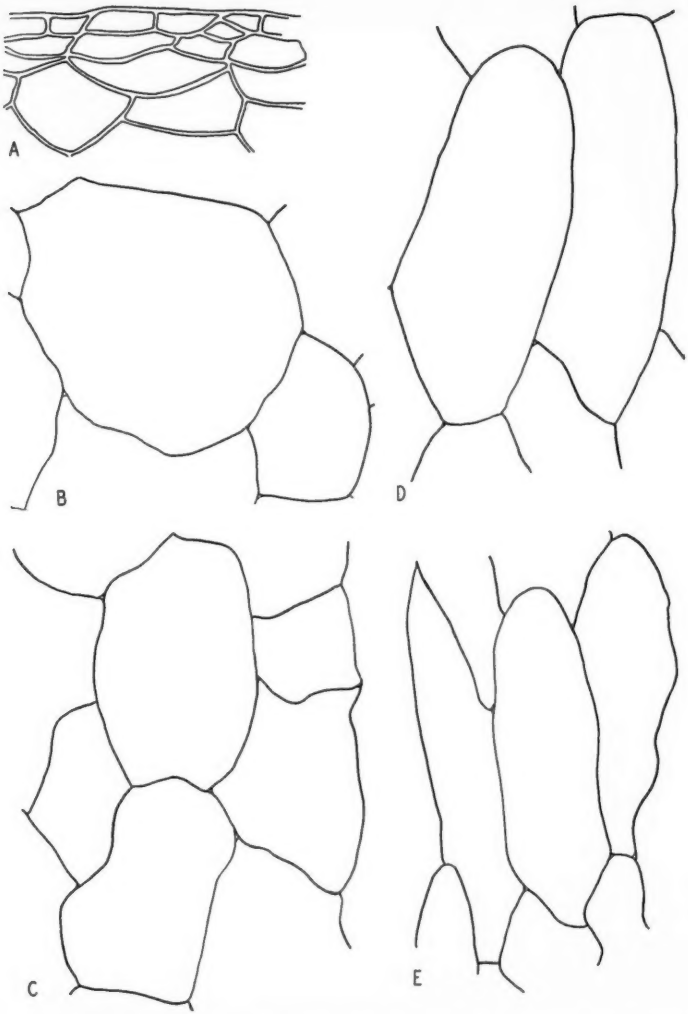


FIG. 7.—Size and shape of cells involved in final swell of fleshy pericarp in radial direction through cheek of sour cherry at fruit ripening (end of stage III), showing transverse elongation in epidermis and hypodermal layer (*A*); roundish shape in next underlying region between hypodermal layer and ring of vascular bundles (*B*); oval shape and beginning of radial elongation in next underlying region (*C*); long oval shape in next underlying region (*D*); and decided radial elongation in inner region (*E*). Inmost layer of small cells adjacent to stony pericarp not shown (camera lucida ×140).

that it represents only the actual increase of the tissues of the ovary wall, whereas figure 1 also includes the ovarian cavity besides both sides of the ovary wall. Since the increase in the diameter of the ovarian cavity during stage I does not involve a corresponding increase in tissue, the steepness of the curve in stage I of figure 8 is much less than that of figure 1. The figure does show graphically the relation between cell division and cell enlargement and the part played by both in the growth of the cherry fruit.

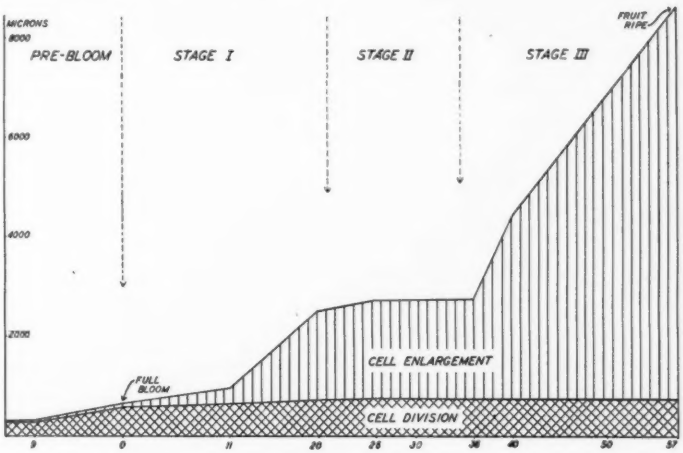


FIG. 8.—Increase in thickness of ovary wall of sour cherry at cheek, from pre-bloom to fruit ripening, showing amount due to increase in number of cells and amount due to enlargement of cells already present. Vertical lines = size changes due to cell enlargement. Cross hatching = size changes due to cell division.

The similarity of development of the sour cherry to the development of other drupe fruits has been noted. The studies of TSCHIERSKE (**19**) with the red raspberry are particularly noteworthy. He observed two periods of rapid development in the drupelets: a first one early in the season during which cell division mainly occurs, and a second just before fruit ripening, in which stretching of the cells occurs. Particularly interesting is his observation that the final swell begins when the structures of the seed and surrounding tissues are differentiated, as noted for the cherry and peach by TUKEY (**21, 22**).

At maturity the fruit bears a close resemblance to the descriptions given by TSCHIERSKE (**19**) for the drupelets of *Rubus ideaus*, by FARMER (**8**) for *R. fructicosus*, and by WINTON (**24**) for *R. strigosus* and *R. occidentalis*. Further, WINTON mentions a striking similarity between the red raspberry and the peach.

Summary

1. This paper pictures and discusses the gross development of the fruit of the sour cherry (*Prunus cerasus* L. var. Montmorency) from 18 days before full bloom to fruit ripening, and the histological changes during the pre-bloom stage, stage I (rapid development for 20 days following full bloom), stage II (retarded development for 16 days), and stage III (rapid development for 21 days to fruit ripening).

2. Three principal tissues compose the ovary wall: inner and outer epidermis, stony pericarp, and fleshy pericarp. The stony pericarp may be divided into an inner and an outer layer, and the fleshy pericarp into an innermost layer of small thin-walled parenchyma, a middle region of large thin-walled parenchyma, and an outer or hypodermal layer of collenchyma.

3. The fleshy and the stony pericarp are derived each from distinct groups of cells which are early separated from one another by characteristic size, shape, and frequency and periodicity of cell division.

4. The inner layer of the stony pericarp is derived from the inner epidermis, together with a few adjacent cells of the pericarp, and forms a band or "hoop" of transversely elongated cells bounding the inner ovary wall. The outer layer is derived from the pericarp, and the cells of which it is composed are elongated at right angles to those of the inner layer.

5. The cells of the stony pericarp increase in number during the pre-bloom stage and the first few days of stage I, and enlarge during the latter part of stage I. Cell walls become progressively thicker, and by the end of the period the maximum number and size of cells is attained. During stage II the walls thicken and harden greatly. During stage III there is slight increase in hardness and brittleness.

6. The cells of the fleshy pericarp increase in number during the pre-bloom stage and the first half of stage II. In the last half of stage II they double in diameter. During stage II there is slight enlargement. During stage III the size of individual cells increases remarkably. Those of the hypodermal layer become enlarged in a tangential direction similar to the adjacent cells of the outer epidermis. Those in the outer portion of the fleshy pericarp become roundish oval, with the greatest diameter parallel to the periphery of the fruit; those next inward become roundish; those next, obovate in a radial direction; next, radially elongate; and innermost, decidedly radially elongate. At maturity the largest cells indicate an increase of twenty-five times in diameter from the size at full bloom.

7. The epidermal cells are elongated radially 18 days before full bloom. They increase rapidly in number during the pre-bloom stage and during the first half of stage I, increase in size and wall thickness during the latter half of stage I, change but little during stage II, and greatly enlarge tangentially in stage III.

8. The stomata are fully differentiated 18 days before full bloom. The guard cells increase in size as the fruit develops, but the increase is less than that of typical epidermal cells.

9. The similarity of other fruits and the mechanism of enlargement in stage III are discussed.

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PLANT SUCCESSION ON GRANITE ROCK IN EASTERN NORTH CAROLINA

HENRY J. OOSTING AND LEWIS E. ANDERSON

(WITH NINE FIGURES)

Introduction

A few studies of the development of vegetation upon bare rock have been made in widely separated sections of the country. These show that the progression of growth forms and even of certain genera is remarkably similar everywhere. Following our study[1] of a mountain exposure in North Carolina, it seemed logical to make a comparative study of lowland outcrops. Several outcrops were located on or very near the fall line which marks the transition between the Coastal Plain and Piedmont provinces.

The transition between the Coastal Plain and the lower Piedmont is usually gradual, but there is a definite change in level. Along the fall line the higher lying rock of the Piedmont is frequently exposed in areas of varying sizes up to several acres. In North Carolina these outcrops are most abundant and extensive northeast of Raleigh in Wake and Franklin counties, but lesser ones appear intermittently toward the southwest. In a comparable physiographic position in South Carolina there are extensive outcrops in Lancaster County, and in Georgia they are both numerous and widespread.

The rock is granitic and weathers slowly. The surface is smooth and usually free of crevices, while the general relief is interrupted only by minor depressions and irregularities (figs. 7, 9). Several areas are crossed by streams and others have a slope sufficient to cause vigorous washing by heavy rain.

The annual rainfall for the region varies from 45 to 46 inches, most of the precipitation occurring in winter and spring. Summer is characteristically hot, with extended dry periods. The resulting forest types are typical of a large portion of the southeastern states. Oak-hickory forests occupy old undisturbed sites, while loblolly

[1] OOSTING, H. J., and ANDERSON, L. E., The vegetation of a barefaced cliff in western North Carolina. Ecology 18:280–292. 1937.

(*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) grow in abandoned fields and cutover land. The poorer sites support Virginia pine (*P. virginiana* Mill.) or post oak (*Quercus stellata* Wang.) and black jack oak (*Q. marilandica* Muench.). The latter are the usual species near the outcrops, indicating that the soil mantle is probably thin for some distance around the exposed rock.

Conditions for plant growth on exposed rock are obviously extreme. The habitat requires adaptations, resulting in a flora that is duplicated nowhere else, and many species of plants so adapted are found growing only on rock. Their ranges are accordingly determined by the geographical occurrence of the rock exposures, and for these reasons may well be considered as rock endemics. The hot dry summers of the southeastern states further accentuate the factors controlling rock vegetation, and result in an especially large number of rock endemics for the section.

The richest flora is found in early spring when temperature and moisture are most favorable. Several species appear then in great abundance, but with the coming of summer they soon disappear and the entire aspect changes. Only the true dominants remain. Especially in the early stages of succession the dominant species are few. All are low growing and mat-forming.

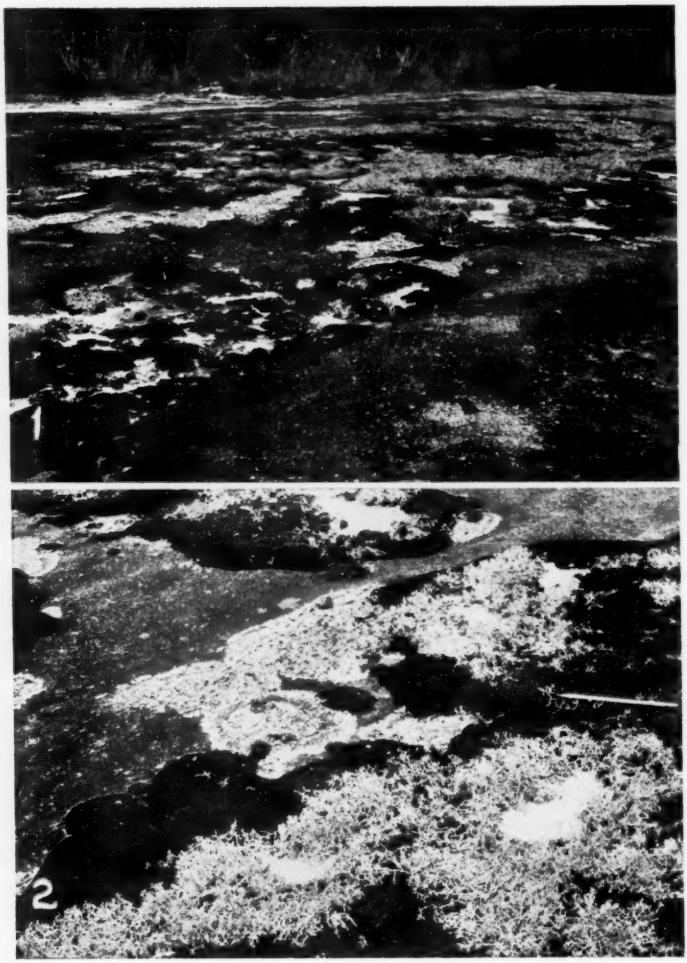
Such a widely extending special habitat, capable of supporting numerous species with restricted ranges, deserves further botanical consideration.

Succession

It is necessary to distinguish two surface variations which determine succession upon the bare rock. Each has a characteristic vegetational development and each materially affects the rate of change. These variations are (1) the dry bare rock surface in general, and (2) the depressions below the level of the surface proper.

Rock surface

Pioneers may appear wherever a minute crevice or roughened surface allows the plants an anchorage. Whether the surface is flat, undulating, or steep (as on the slopes of some of the domed outcrops), the vegetation is much the same everywhere. Moisture can be retained only by mat-forming species and then only for a short



FIGS. 1, 2.—Fig. 1, pioneer stages of mat formation on bare rock, showing mats of *Grimmia* and of *Grimmia* and *Cladonia*. Fig. 2, detail of *Grimmia* spreading on bare rock with *Cladonia* invading the mat. Note small patches of former beginning on bare rock.

time. The pioneers may be desiccated for long periods at any season of the year. Change is extremely slow and in some places may actually be in a condition of pioneer equilibrium.

The earliest pioneers are uniformly the same on every rock area studied, regardless of the surface of the rock. *Grimmia laevigata* (Brid.) Brid. is regularly the pioneer mat former (fig. 1). Its habitation of the rock is definitely not restricted to areas occupied by crustose lichens or by other previous invaders (figs. 1, 2). Crustose lichens such as *Verrucaria nigrescens* Pers. are perhaps the first plant occupants, but they accumulate so little soil as to be of no importance in aiding later plants to become established. This is also true of *Parmelia conspersa* (Ehrh.) Ach., a foliose form which, although present in restricted patches on all the rock, never shows evidence of being replaced by other species (figs. 2, 4).

Small tufts of *Grimmia*, once established, spread apparently by vegetative means and may form mats of considerable extent before being invaded by other species (fig. 1). These mats of *Grimmia* catch and retain a comparatively large amount of mineral soil which blows or washes over them. Soil often accumulates until only the tips of the plants are visible and the mats may be 1 to 3 inches deep. Associated with *Grimmia* when considerable mineral soil is held in the mats is the small succulent *Diamorpha cymosa* (Nutt.) Britton. Although in spring it may occur so thickly as to obscure the *Grimmia*, it disappears in summer and is relatively unimportant in aiding further development of the mats. *Diamorpha* may be considered merely as an associate of *Grimmia* growing in the mineral soil in a situation free from competition with larger species which are unable to survive in such severe conditions.

The pioneers at the periphery of a mature mat are continually spreading upon the rock. At the same time new species are invading the center of the mat, which is the oldest portion. These invaders advance centrifugally over the mat at about the same rate that the pioneers spread upon the rock, and they may themselves be superseded by other species which again invade the central area. This results in a series of more or less concentric girdles, each representing a stage in mat development. The pioneer stage is invariably at the periphery, the most mature at the center. There are numerous

species associated with these stages, but their relationships are clear when considered in the rather distinct girdles. Each girdle usually has but a single dominant. The other species are seasonally important or may merely be associated with the dominant because of the conditions it provides. The dependent species contribute little to the development of the mats.

Successional development of a *Grimmia* mat comes through invasion by *Cladonia leporina* E. Fries and rarely by *C. caroliniana* Tuck. (fig. 2).[2] The lichens spread from the center of the mat at about the same rate that *Grimmia* is advancing. The lichens, being coarse and large, add considerably to the thickness of the mat, facilitating the collection of soil. At the same time or shortly after the appearance of *Cladonia*, *Selaginella rupestris* (L.) Spring invades the mat, whereupon mixed growths of *Cladonia leporina* and *Selaginella rupestris* result (fig. 3*c*). These species, being coarser and much larger than *Grimmia*, contribute far more humus and offer a greater resistance to wind- and water-borne debris. Thus with their invasion the rate of succession is accelerated. *Arenaria brevifolia* Nutt., especially conspicuous in spring (fig. 4), is associated with this girdle. When desiccation begins it entirely disappears. Although it may be present on *Grimmia* mats, it reaches its maximum development with the *Selaginella* stage.

With the deepening of the mat due to both mineral and organic additions, *Polytrichum ohioense* Ren. & Card. makes its appearance (fig. 3*d*). *Selaginella* and *Cladonia* are soon completely excluded. Here again are conspicuous associates which probably affect the mat only slightly. *Crotonopsis elliptica* Willd. and *Hypericum gentianoides* (L.) BSP., although growing elsewhere in some abundance, are here consistently present and evenly distributed.

Conditions for growth are now apparently comparable with those of some abandoned fields in the vicinity, for a weed complex appears that is similar to the vegetation in fields during the early years of their abandonment. In addition to *Hypericum*, *Diodia teres* Walt., *Ambrosia artemisiifolia* L., and *Panicum philadelphicum* Bernh. are

[2] We acknowledge with thanks the cooperation of Dr. ALEXANDER W. EVANS, who identified the Cladoniae mentioned in this study.

initiates and *Geranium carolinianum* L., *Trifolium procumbens* L., *Krigia virginica* (L.) Willd., *Linaria canadensis* (L.) Dum., *Gnaphalium purpureum* L., and *Cerastium vulgatum* L. are typical later

FIG. 3.—Margin of mature mat showing successive girdles of *Grimmia*, *Cladonia*, *Cladonia* and *Selaginella*, and *Polytrichum*.

members. Eventually, as in old fields, *Andropogon virginicus* L. and *A. ternarius* Michx. become the dominants (fig. 7), with *Eupatorium hyssopifolium* L., *E. rotundifolium* L., *Senecio smallii* Britton, *Salvia lyrata* L., and *Panicum xalapense* HBK. as associates. Especial men-

tion should be made of *P. columbianum* var. *thinium* Hitchc., since it has been found nowhere in North Carolina except on these outcrops.

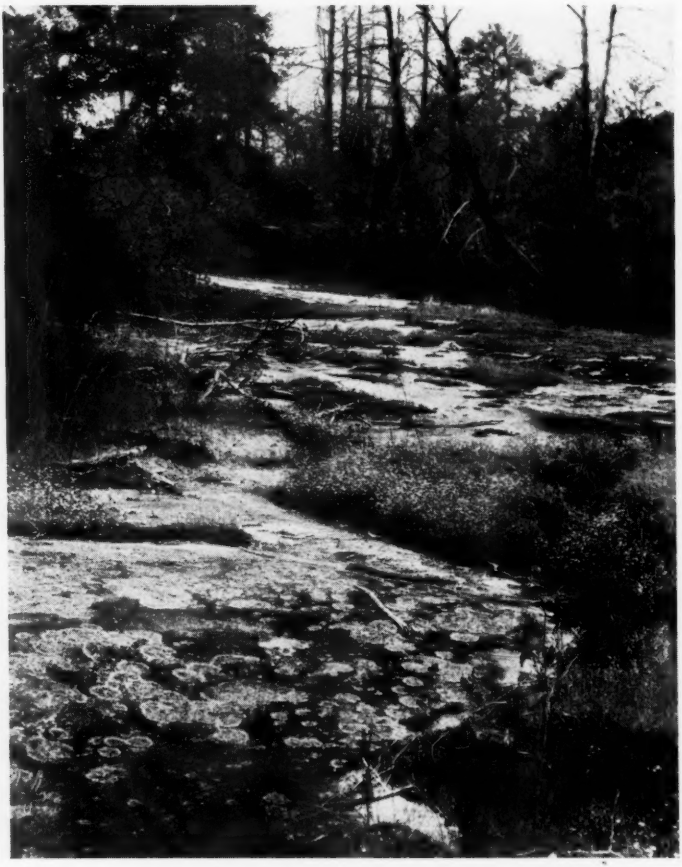


FIG. 4.—Margin of mat in spring. Girdles obscured by mass of *Arenaria*. Note large *Juniperus virginiana* on mature mat at left and dead pines in background.

If given time, such a mat thickens and develops a soil layer capable of supporting certain woody seedlings which germinate upon it. *Juniperus virginiana* L. is a typical early tree species (fig. 4),

with such drought resistant shrubs as *Rhus copallina* L. and *Batodendron arboreum* (Marsh.) Nutt. as associates (fig. 8). *Pinus virginiana* or *P. echinata* is apt to follow if seed trees are near.

The tree stage is nowhere well developed. Mats are never sufficiently extensive to support more than a few trees and some of them invariably are dead (fig. 4). The scrubby habit and slow growth rate of the woody species indicate the unfavorableness of the habitat.

Depressions

The regularity of the rock surface is interrupted here and there by depressions, which vary in depth and size. The associated vegetational effects are caused by the water, which after every rain is retained until evaporated or used by the plants. On the basis of depth and drainage, there may be dry depressions, moist depressions, or pools, each with a distinctive vegetational development.

Dry depressions.—Most of the depressions are shallow and flat bottomed, the amount of water held being determined by the level of the lowest part of the rim. When a portion of a rim is eroded, the depression may retain only a thin layer of water, which in summer evaporates soon after it has fallen. The rim of the depression serves to obstruct and retain transported soil, which forms a level floor of silt and sand that bakes hard and dry after every rain. Such depressions are perhaps less favorable habitats than the bare rock itself. The layer of soil is too thin and bakes too hard to support most species, while its depth excludes the bare rock pioneers. Only succulents or small wiry species seem capable of growing in this extreme situation (fig. 7). Most of the latter are seen only in spring, leaving the flats almost completely bare in summer. *Talinum teretifolium* Pursh. is rather regularly present, as is *Portulaca smallii* Wilson. If the soil is sufficiently deep *Diamorpha cymosa* is often abundant. Mineral soil and absence of competition seem to constitute the requirements for these rock succulents. Any of them, and especially *Diamorpha*, occur as associates of *Grimmia* when an appreciable mineral layer has been formed. *Arenaria brevifolia*, a delicate annual growing only in early spring, is a rock species which, like *Diamorpha*, requires a mineral soil of a favorable depth and a minimum of com-

FIG. 5.—Shaded tongue of rock at edge of woods, with *Entodon* growing on organic debris.

petition. Here and there a sandy hollow may produce a solid stand of *Arenaria*, which dies as the depression dries out.

Other non-succulents surviving on the baked soil are *Fimbristylis baldwiniana* (Schult.) Torr., *F. autumnalis* (L.) R. & S., *Stenophyllus*

FIG. 6.—Rock pool with characteristic raised ring of pioneers around margin. Behind pool, a moist depression approaching *Andropogon* stage.

capillaris (L.) Britton, *Cyperus inflexus* Muhl., and *Agrostis elliottiana* Schult., the last being a southern species restricted to these rock outcrops in North Carolina. Such dry depressions usually give no evidence of further successional development. It is probable that many have long been in this condition and will so remain unless the contours of the marginal rock change. Others, comparable except

for a slightly deeper layer of soil, may be occupied by masses of *Diodia teres*, *Crotonopsis elliptica*, *Hypericum gentianoides*, and even a few clumps of *Polytrichum commune*. *Lechea racemulosa* Lam. with *Eupatorium pinnatifidum* Ell. represent an intermediate stage in deep sandy depressions. Indicative of the better moisture conditions is the *Andropogon* stage which follows and the fact that the shrubs include not only *Rhus copallina* and *Batodendron arboreum*, but also scattered plants of *Rubus* spp. In these deeper depressions filled with mineral soil the mature stages frequently include seedlings of *Liquidambar styraciflua* L., and still older areas which may have had a similar origin support *Pinus taeda* and an occasional *Quercus stellata* (fig. 9).

MOIST DEPRESSIONS.—Moist depressions are intermediate as to moisture, and retain some water after every rain but not enough to support species with aquatic or marsh characteristics.

Succession in the undrained depressions is less obvious and more irregular than on bare rock. The depth of water varies with the depth of the hollow and fluctuates with the supply and rate of evaporation. Few species can endure alternate periods of complete inundation and prolonged desiccation. This is the situation in most of the depressions, for few are sufficiently large or deep to be classed as permanent pools.

As elsewhere on the rock, *Grimmia laevigata* is the pioneer in the moist hollows. Depending apparently upon moisture conditions, it is followed by *Cladonia leporina*, *C. caroliniana*, or *C. tenuis* (Floerke) Harm. Occasionally all three species may simultaneously invade a mat of *Grimmia*. These species retain soil and debris efficiently and soil accumulates much faster in the depressions than on the flat surfaces already described. The accumulation of soil and the moist condition facilitate the growth of *Polytrichum ohioense*, which next invades the mat. In the moister mats *Selaginella* is not present, for it either cannot withstand the moist conditions or it is excluded by the luxurious growth of *Polytrichum*. Large tufts of *Campylopus introflexus* (Hedw.) Brid. are at times mixed with *Polytrichum*. When the moisture conditions are especially favorable the *Polytrichum-Cladonia-Grimmia* mats often attain depths of 6 to 10 or more inches.

FIGS. 7–9.—Fig. 7, general view of typical flat exposure. Moist depression in *Andropogon* stage (center and right foreground) and dry, sand-floored depression (left foreground) supporting *Stenophyllus*, *Talinum*, and *Portulaca*. Fig. 8, mat in shrub stage. *Rhus copallina* in center surrounded by girdle of *Andropogon*, then *Polytrichum* obscured by *Crotonopsis* and *Hypericum;* at very margin, advancing girdles of *Cladonia* and *Grimmia*. Fig. 9, old mat of depression origin supporting pines and hard-woods. Several trees dead.

After the early mat-forming stages, the moist hollow succession soon becomes rather like that on bare rock. The mat spreading over the rock at the margins of the depression has, of course, the same characteristics as one originating on the bare rock. The portion of the mat over the depression is thicker than a bare rock mat and has other species because of better moisture conditions. Added species typical of mats originating in depressions include *Rumex verticillatus* L., *Sphenopholis obtusata* (Michx.) Scribn., *Panicum oricola* Hitchc. and Chase, *Galium claytoni* Michx., *Trisetum pennsylvanicum* (L.) BSP., *Myosotis virginica* (L.) Link, and *Specularia biflora* (R. & P.) F. & M. The two lines grade into each other, therefore, and the central portion of a mature mat must be examined to identify its origin. An abundance of *Rubus* spp. in the early shrubby condition and the regular presence of *Ulmus alata* and *Liquidambar styraciflua* offer rather positive evidence of depression origin. Except for minor variations the species and their relationships are much the same in the later stages as those described for bare rock.

POOLS.—Any depression which supports aquatic or marsh species may be termed a pool. Except after prolonged drought, there will always be mud in the bottom, if not standing water. Few pools are sufficiently large or deep to be permanent.

The regular rock and depression species previously discussed develop around the margins, spreading over the rock and also advancing centripetally as the water level permits. The moisture speeds growth and frequently the mat formers are present as a raised ring almost completely circling the pool (fig. 6). The marginal ring has a development comparable with that of damp depressions and invariably becomes dominated by *Polytrichum ohioense*. The ring is fringed at the water's edge by *Philonotis fontana* Brid., *Bryum bimum* Brid., *Climacium americanum* Brid., and *Aulacomnium palustre* Schwaegr. Toward the dry rock are two girdles comparable with rock succession. The *Polytrichum* grades into *Cladonia caroliniana*, with *Hypericum gentianoides* and *Crotonopsis elliptica* associated, and the outer margin is a *Grimmia* girdle with its usual associates of *Arenaria brevifolia* and *Diamorpha cymosa*, and the frequent addition of *Talinum teretifolium*. Obviously this is the normal or moist depression series spreading from the margin of the pool.

If there is no soil in the depression, the development of vegetation in the water is delayed until mineral and organic materials cover the bottom. Even then development is slow, because the pool periodically becomes dry and aquatic species may not survive. The shallower, mud-bottomed pools with little or no vegetation invariably support *Lindernia monticola* Muhl. and less frequently *Utricularia juncea* Vahl. Deeper pools are often filled with a dense tangle of *Proserpinaca palustris* L. If soil is accumulating, *Gratiola neglecta* Torr. may occupy the muddy margin, while *Eleocharis obtusa* (Willd.) Schultes, *Juncus effusus* L., and sometimes *Typha latifolia* L. form emergent clumps in the water. As these species increase they accelerate soil building and the habitat tends to become a marshy depression rather than an open pool (fig. 6).

The dominants are the Cyperaceae, including ten species of *Carex* and several species of *Cyperus*, none of which are constantly present. *Rynchospora microcarpa* Baldw., *R. glomerata* (L.) Vahl., and *R. cymosa* Ell. may be found on every outcrop where pools are filling up. *Scleria pauciflora* Muhl. is frequently present, as is *Juncus acuminatus* Michx. Other species occurring rather generally are *Seriocarpus linifolius* (L.) BSP., *Lobelia nuttallii* R. & S., *Xyris ambigua* Beyr., *Ranunculus pusillus* Poir., *R. abortivus* L., and *Chaerophyllum tienturieri* Hook.

Shrubs appear only after a long period of the marsh condition and after the soil has been gradually built up to the level of the general rock surface. If the site remains fairly moist, *Alnus rugosa* (DuRoi) Spreng. may be an early woody species. The marshy condition may persist for a long time, supporting deep beds of *Polytrichum* and even *Sphagnum* long after woody species have gained a foothold. Intermediate moisture conditions are indicated by *Rubus*, followed by *Liquidambar styraciflua* and *Ulmus alata*.

Marginal vegetation

The borders of the more extensive outcrops are exceedingly irregular in outline. The vegetation along the margins is for the most part stable and shows little evidence of migrating upon the rock itself. Woody species usually extend to within a few feet of the edge of the rock.

Some margins are almost continuously moist because of seepage from the adjoining forest. In these seepage zones water accumulates in the outer layer of soil and vegetation, resulting in an especially luxuriant fringe of bryophytes and small herbs which have a characteristic boggy aspect. Here are deep beds of *Sphagnum recurvum* Beauv., *S. subsecundum* Nees., and *S. imbricatum* Kornsch., with *Climacium americanum*, *Aulacomnium palustre*, *Leucobryum glaucum* Schimp., and *Polytrichum commune* Hedw. If very moist, *Bryum bimum* and *Philonotis fontana* are included. Scattered on the bogs are *Danthonia sericea* Nutt., *Alopecurus carolinianus* Walt., *Veronica arvensis* L., *V. peregrina* L., and *Selaginella apoda* (L.) Fernald. Beyond the bog, along the mineral margin, the typical *Selaginella-Polytrichum* zone may be found to grade into *Andropogon* and shrub zones adjoining the forest.

Where irregular tongues of bare rock extend back into the forest the margin is shaded and protected from washing rains (fig. 5). Here loose organic debris from fallen branches and twigs accumulates. The organic material is soon occupied by *Entodon seductrix* (Hedw.) C. Muell., which forms a loose and insecure covering extending as far out on the rock as the overhanging branches of the trees. Permanent establishment is difficult because the *Entodon* is not anchored to the rock. Even though protected, the mat is periodically disturbed and only a narrow margin is stable enough for other plants to invade it. Some of these are *Thuidium delicatulum* (Hedw.) Mitt., *Dicranum scoparium* Hedw., *Cladonia strepsilis* (Ach.) Vanio., *Oxalis stricta* L., *Talinum teretifolium*, *Woodsia obtusa* (Spreng.) Torr., *Geranium carolinianum*, *Asplenium platyneuron* (L.) Oakes, *Cerastium vulgatum*, *Saxifraga virginiensis* Michx., and occasionally large tufts of *Cladonia tenuis*. The vegetation of the shaded, protected margins may migrate to some extent, depending upon how successfully anchored the *Entodon* complex becomes. There is evidence that some of these protected spots may eventually become filled in with vegetation and thus even out the irregular outline of the margin.

Not all of the rock outcrops are closely surrounded by trees. In level country a few are bordered by cultivated fields, which must at one time have been wooded. Only on the open outcrops is there

evidence of a migration of marginal vegetation upon the rock. The succession is as described for "rock surface." The occurrence of marginal migration in the shadeless areas offers a clue to its absence in wooded areas where the margins are shaded for a large part of the day. *Grimmia*, which is the only mat pioneer, is intolerant of shade, and succession cannot be initiated along wooded margins. Since *Grimmia* grows only on dry, well drained surfaces, the frequent seepage areas of the shaded margins undoubtedly further exclude it because of excess moisture.

Discussion

The rock of the outcrops is similar in all localities. The outcrops occupy the same geographic position everywhere, so that the causes of their exposure must have been similar. Since the exposures are irregularly distributed along the fall line in at least three states, they must (at least indirectly) owe their origin to factors which produced the fall line.

The exposures have been in their present condition for a long period. Certainly if they originated with the fall line there has been sufficient time for vegetation to have developed a complete cover and to have produced a well bound soil mat over the largest of them. Although growing conditions are extreme and the rate of succession is slow, apparently a cover can develop on any of the exposures. It is not unreasonable to suppose that at one time the rocks were completely covered with at least sufficient soil to support a meager forest. With the advent of the white man, lumbering, fires, and erosion might well have exposed these areas where the rock was nearest the surface.

For some distance surrounding an exposure the rock is covered with a comparatively thin layer of soil, usually unsuited to agriculture. These poor sites may not support dense or well developed forest stands but almost invariably they are forested, indicating that in recent years man's disturbances of the rock margins have been only minor. The reasons for the lack of cover on the rock surfaces today must be sought elsewhere.

No mats are found which support more than a few individuals of tree size. These larger individuals are always inferior specimens and

frequently die before attaining much growth. Twenty-five years is a good average age for the larger trees on the mats. It is apparent that somehow the development of the mats is checked as they approach maturity.

Two factors probably tend to restrict the further development of the older mats. Since the trees are rooted only in the mats they have no substantial anchorage. The larger they become the more they are subject to wind-throw. Down trees are the rule on every outcrop. When a tree is upturned, its roots raise the mat with them and may mean almost its complete destruction. The second factor is fire. The numerous down trees, the highly organic mats, and the extremely dry condition of the vegetation during summer all provide ideal conditions for almost clean burning when a fire starts. In the general region of the outcrops summer fires are common. Fire protection is concentrated not on these worthless rock barrens but on more valuable properties. Consequently every outcrop has evidence of fairly recent fire, and invariably the area burned is reduced to a bare rock condition except where depressions hold mineral soil. Perhaps these two factors are sufficient to explain why the outcrops remain barren. Erosion, which must have played its part in their early exposure, is today of importance only on a few steep slopes or locally on a portion of an outcrop.

This is in contrast with the mountain exposures in western North Carolina. These are on steep slopes where an enlarging mat, unless well anchored, is doomed because its own weight will release it. Fires are infrequent and precipitation regular throughout the growing season. Here then, erosion and gravity are the factors which check the unlimited development of the mats.

The two types of exposures are roughly in the same latitude but separated some 3000 feet in altitude. This altitudinal difference results in a much shortened growing season in the mountains. However, temperatures are not so high, complete desiccation is rare, and almost continuous summer growth is possible because of the frequent mountain rains. In spite of these differences the growth forms, and even genera, are remarkably similar in their successional relationships. However, species are only rarely duplicated. It should be noted that in neither habitat do crustose lichens play any important

part in the development of later stages of vegetation. They are pioneer species in both climates, but rarely, if ever, are they followed by other forms. Xerophytic mosses comprise the pioneer community which, coming in on bare rock, begins mat formation and succession. The crustose lichens are merely incidental to the normal line of development, neither hindering nor contributing to it.

Summary

1. The transition between Piedmont and Coastal Plain provinces, called the fall line, has numerous outcrops of granitic rock scattered along its length throughout North Carolina, South Carolina, and Georgia.

2. Rocks are unfavorable habitats for plant colonization and these are particularly so because of the long dry summers with high temperatures. Especially adapted plants, including several rock endemics, grow here in spite of the extreme conditions. Succession on the North Carolina rocks follows two major lines, originating (1) anywhere on the rock surface and (2) in depressions. The depressions may be dry, moist, or pools.

3. The bare rock surface is invaded by *Grimmia* or crustose lichens. The latter do not contribute to further succession. *Grimmia* forms mats which are successively invaded by stages dominated by (1) *Cladonia-Selaginella*, (2) *Polytrichum*, (3) *Andropogon*, (4) conifers.

4. Dry depressions retain little water and are floored with a clayey soil. The soil is too thin to support species which lead to a successional series and too deep to permit the beginnings of a normal rock succession. They support scattered succulents like *Talinum* and *Portulaca*, and sometimes dry, fibrous individuals like *Fimbristylis* and *Stenophyllus*.

5. Damp depressions represent the best development on the rocks. The stages are essentially like those on the rock surface but the additional moisture results in richer and more rapid growth, with hardwoods appearing in the later stages.

6. Pools are usually intermittent but regularly support marsh species. The margins have the normal rock series spreading from the pool and Cyperaceae predominate among the soil builders in the

water or mud. Late stages are dominated first by mesophytic shrubs and later by hardwoods.

7. Margins are irregular. Where seepage occurs there may be small boggy areas dominated by *Sphagnum*. Dry margins are usually fixed and rarely show evidence of migration upon the rocks. Shaded margins, protected from washing, produce a distinctive pioneer community upon the debris which accumulates. *Entodon* is the dominant.

8. The exposures must owe their origin to factors which caused the fall line. Considering the time since this line was formed and the rate of plant succession, it is proposed that the rocks were once entirely covered although probably not so richly forested as the surrounding country. The activities of man resulted in repeated fires and erosion which again uncovered the rock, and today drought, wind-throw, and fire undoubtedly maintain them in their semi-barren condition.

DEPARTMENT OF BOTANY
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STRUCTURE OF SOME CARBONIFEROUS SEEDS FROM AMERICAN COAL FIELDS

FREDDA D. REED

(WITH TWENTY-SEVEN FIGURES)

Introduction

The early accounts of seeds or seedlike fructifications from the Paleozoic rocks of America, while made chiefly from casts and impressions, nevertheless demonstrate wide variation in form and size and consequently indicate a multiplicity of species. DAWSON, in his geological studies in Acadia (**8, 9, 10, 11, 12**), recognized sixteen species distributed in four genera. Although his descriptions are valuable from a taxonomic point of view, yet, as shown by STOPES (**29**) and from a reinvestigation of *Trigonocarpon hookeri* reported here, his interpretations—particularly of any cellular structure found therein—are often unreliable and even erroneous.

The reports of LESQUEREUX (**18, 19**), NEWBERRY (**20, 21, 22, 23**), WHITE (**30, 31, 32**), NOÉ (**24**), ARNOLD (**2**), DEEVERS (**13**), and others (**1, 5, 14**) greatly increased the number of species and added new terrains, until at the present more than 170 seed species have been figured, collected from the Upper Devonian and Carboniferous strata of North America, and geographically from Nova Scotia through the coal fields of eastern United States and west to Arkansas.

More recently the researches of KRICK (**17**), GRAHAM (**15, 16**), ARNOLD and STEIDTMANN (**3**), and DEEVERS (**13**), on the anatomy of seeds found in coal balls have not only contributed to our knowledge of their structure but have shown that there is variation in histological detail, thereby justifying in part the numerous species formerly reported. Owing to imperfection of fossilization and the age of the ovule when detached from the parent plant, superficial characters of form and size become of equivocal value in establishing new genera and even new species; but when variation in these characters is supported by variation in anatomical detail, then the existence of a multiplicity of species must be admitted.

Despite the number of seeds that have been investigated, no completely preserved specimens have been found in carboniferous strata; so incomplete are they that their exact nature is not known, as indicated by the fact that they have been variously referred to as fruits, nuts, ovules, seedlike, and seeds (**17**). Since information concerning their morphology becomes cumulative, each and every specimen retaining any cellular detail assumes an importance in helping eke out the story of their structure and evolution.

Observations

Conostoma oblongum Will.

The genus *Conostoma* was founded by WILLIAMSON (**34**) in 1877 to include three small seeds of the order Lagenostomales. The seed specified *C. oblongum* was found in the Lancashire coal fields, which belong in the Lower Coal Measures. In 1911 OLIVER and SALISBURY (**27**) published a detailed account of *C. oblongum* based upon an assemblage of all the known seeds of the species collected subsequent to WILLIAMSON's description. The comparatively few specimens of the assemblage, sixteen in all, led OLIVER and SALISBURY to state: "*Conostoma oblongum* is one of the rarest of the Paleozoic seeds." In 1932 KRICK (**17**) described briefly some specimens found in coal ball 213 of the Harrisburg, Illinois, collection. The incomplete preservation of her material, however, together with the obliquity of the preparations, made the seeds of little more value than to serve the purpose of identification and to report a new geographic origin and geologic stratum for the genus.

In addition to *C. oblongum*, other species of the genus have been described. Two of the three species upon which WILLIAMSON founded the genus, *C. ovale* and *C. intermedium*, were later reinvestigated by BENSON (**4**), who transferred them to another genus, *Sphaerostoma*, and reduced them to one species, *ovale*. OLIVER and SALISBURY established a second species, *C. anglo-germanicum*, for the reception of some specimens from Shore, Littleborough, England and from Duisburg in Rheinpreussen, Germany. GRAHAM (**15**) determined two more species, *C. platyspermum* and *C. quadratum*, found in coal balls from the Calhoun mine, Richland County, Illinois. The coal balls from this mine are of the same geologic age as

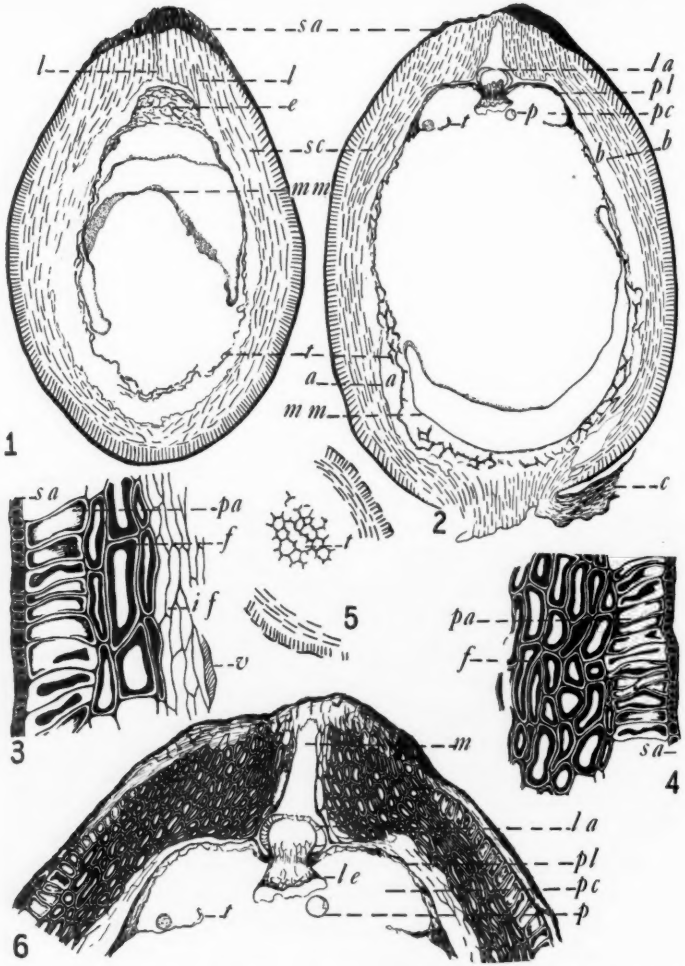
those from Harrisburg, the Allegheny group of the Upper Pennsylvanian. It is interesting to note that the British and European specimens have all been found in the Lower Coal Measures, whereas the American specimens of *Conostoma* have thus far been found in the Upper Coal Measures.

MATERIAL AND METHODS.—The following description is based upon four specimens found in coal ball 210 of the Harrisburg, Illinois, collection. Because of the small size of *C. oblongum*, the usual methods—the preparation of thin sections by cutting and grinding or the making of celloidin peels each with its consequent loss of material—were not applicable. Instead, sections of the coal ball, 1 mm. more or less thick, containing the seeds were ground with fine carborundum powder which served a double purpose, that of slowly abrading as well as maintaining a polished surface. At frequent intervals and as often as desirable the polished surface was coated with a thin film of Canada balsam, then examined under the microscope by means of reflected light and camera lucida drawings made. In such manner the specimen can be studied serially, and such small objects as the pollen grains, which measure about 80 μ in greatest diameter, can be observed in successive planes, making it possible to discriminate superficial markings from internal structure. Moreover, if at any time during the procedure a particularly desirable plane is revealed, that surface can be mounted permanently and the grinding proceed from the reverse side until the section is thin enough to be studied with transmitted light.

DESCRIPTION.—*Conostoma oblongum* is an elongate cylindrical seed about 4 mm. in length and 2.5 mm. in width. Of transverse sections only oblique ones were available, but these showed no evidence of platyspermy nor of ribs.

The seed remains consist of the integument, nucellus, and megaspore membrane. Within the pollen chamber are some pollen grains and at the base of one of the specimens there was some adherent tissue which appeared to be a remaining fragment of the cupule (fig. 2*c*). Save for the one remaining fragment there was no vegetative tissue in the coal ball in organic connection with the seeds.

The integument, which around the body of the seed is about 0.3 mm. thick, consists of three distinct zones termed by OLIVER and



FIGS. 1–6.—*Conostoma oblongum*. Fig. 1, tangential longisection: *sa*, sarcotesta; *l*, loculus containing vascular tissue shown in fig. 8; *e*, epidermis of nucellus; *sc*, sclerotesta; *mm*, megaspore membrane; *t*, tapetal layer of nucellus. × about 20. Fig. 2, nearly median longisection: *la*, lagenostome; *pl*, plinth; *pc*, pollen chamber; *p*, pollen; *c*, fragment of cupule; other lettering as in fig. 1. × about 20. Fig. 3, detail of portion of integument from *a–a* of fig. 2: *pa*, palisade layer of sclerotesta; *f*, fibrous layer of same; *if*, inner fleshy layer of integument showing traces of vascular tissue, *v*. × about 95. Fig. 4, detail of integument from *b–b* of fig. 2. × about 95. Fig. 5, tangential longisection showing surface view of tapetal cells, *t*. × about 16. Fig. 6, detail from micropylar end of fig. 2: *m*, micropyle; *le*, lenticular nucellar tissue. × about 40.

SALISBURY the sarcotesta, sclerotesta, and endotesta or inner fleshy layer.

The sarcotesta is the investing layer and consists of epidermis only. In this material it is poorly defined, both by reason of the dense contents which obscure the limitation of the cell walls and because of the incomplete preservation. Around the body of the seed the epidermis appears to be fairly uniform; in longitudinal section the cells are square to elongate and about 25 μ thick. At the micropylar region the epidermal cells become radially elongated, thereby increasing the thickness of the layer to more than 100 μ (fig. 1).

Underneath the epidermis is the sclerotesta, composed of two types of cells forming two well differentiated layers. The outer layer is of radially elongated, palisade-like cells, 30 μ wide by 120 μ in radial depth (figs. 3, 4). These cells are likewise filled with some organic material, but it is not so dense and opaque as that contained in the epidermis, and instead of appearing black like the epidermis these cells are golden brown. The uniseriate palisade-like cells inclose the inner layer of the sclerotesta, the cells of which are differentiated by being vertically elongated, fibrous, and multiseriate, of three to seven rows (figs. 3, 4). At the micropylar region these cells increase in number, resulting in a perceptible increase in the thickness of the sclerotesta (fig. 6).

Of the three zones of the integument, the inner one shows the least preservation, doubtless because of the structure of the cells, which are thin-walled, parenchymatous, and vertically elongate. Occasional fragments of this tissue are found in which tracheids with spiral to scalariform thickenings are seen. The inner cells of this zone are always in a fragmented condition (fig. 3).

Because of incomplete preservation the vascular system is not intact, yet the remaining fragments of conducting elements are indicative of some facts with regard to its course. From the chalazal end discrete strands pass up through the inner fleshy layer almost to the level of the summit of the nucellus, and then, accompanied by thin-walled parenchyma, they penetrate the fibrous layer of the sclerotesta (figs. 1, 7, 8) in which they terminate. The number of bundles, determined by the loculi in the distal end of the sclerotesta (fig. 1),

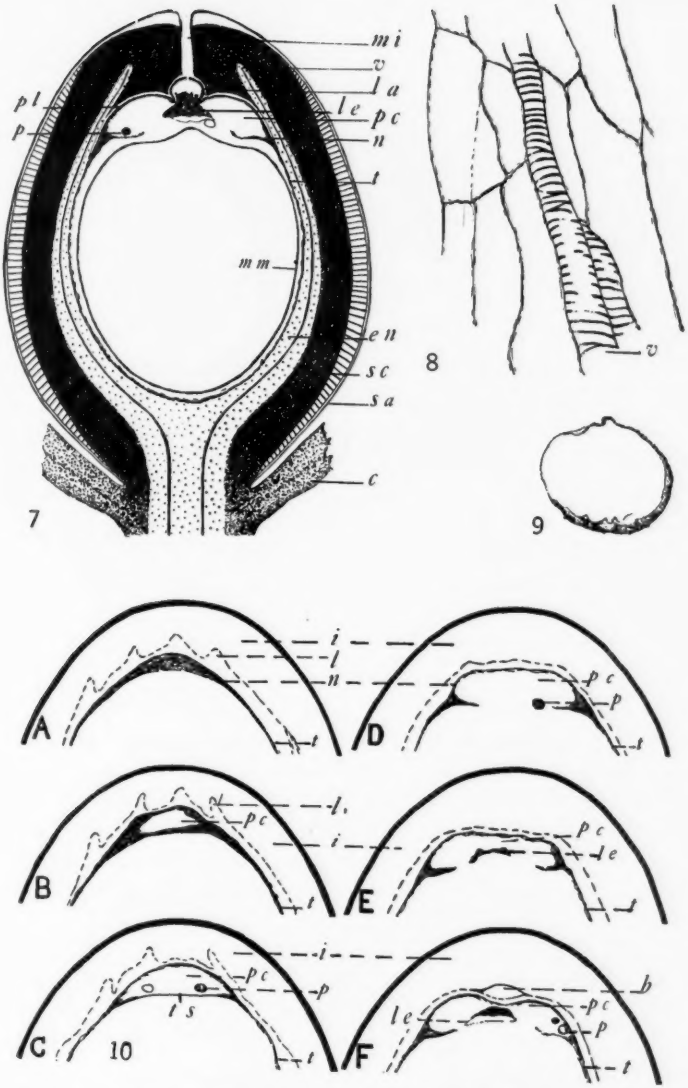
which were formed by disorganization of the soft tissues accompanying the vascular elements, was at least six and probably eight.

The nucellus and integument appear to be nondiverged to the floor of the pollen chamber. Owing to the lack of preservation, the exact point of divergence of the two structures is another moot question.

In their description of *Conostoma*, OLIVER and SALISBURY defined three regions of the nucellus: (1) the main body of the nucellus or megaspore chamber, surmounted by (2) a truncated continuation, the plinth, at the summit of which is a depression in which is (3) the lagenostome. Of the three regions of the nucellus in the specimens under consideration there is little remaining save the limiting layers, which are the epidermis and tapetum (figs. 5, 6). From the point of divergence of the integument and nucellus, the epidermal layer follows the contour of the inner layer of the integument to the base of the micropyle, where it turns in and down, making a depression at the apex of the plinth region. Then, leaving a small orifice about 135 μ in diameter, it expands to form a globular body which fits into the apical depression of the plinth and under the beveled edges of the integument at the base of the micropylar tube (fig. 6). These last epidermal cells are the limiting cells of the lagenostome; while they are continuous with the epidermal cells of the plinth region, yet they differ by the possession of delicate scalariform sculpturing on their walls (fig. 6).

The lagenostome is hollow, and measures about 130 μ deep by 180 μ wide. At the apex there is a small orifice directly under and opening into the micropyle. The lenticular mass of tissue (fig. 6*le*) is not interpreted as being an integral part of the lagenostome but intruded tissue of the plinth, and will be considered later.

The plinth is bounded peripherally by its epidermis, distally is surmounted by the lagenostome, and proximally is separated from the megagametophyte by the tapetal septum. It is about .1 mm. in diameter at the base and about 0.25 mm. deep. The epidermal cells are large and flat and have thicker walls than those of the tapetum. Figure 10 shows a series of somewhat oblique longitudinal sections through the distal end of the seed of successive planes of the plinth from tangential to almost median aspect. In *A* the epidermis of the



FIGS. 7–10.—*Conostoma oblongum*. Fig. 7, reconstruction of median longisection. ×30. Fig. 8, vascular elements from loculus of fig. 1. × about 500. Fig. 9, section of pollen grain found in pollen chamber. × about 500. Fig. 10, series of longisections through micropylar end of seed, showing successive planes of nucellus. ×30.

plinth is seen in surface view; *B* is through the peripheral region of the plinth and pollen chamber; in the plane of *C* the tapetum appears as an unbroken septum over the megaspore membrane and two pollen grains are seen in the pollen chamber; at *D* there is a break in the tapetal septum, which in the next plane, *E*, is capped with tissue that seems to be a single layer of cells of the same texture as those of the tapetum. The progressively more median sections (fig. 10*F*), and figure 6, show this tissue as a lenticular layer with the convex side extending into and plugging the lagenostome. In these specimens of *Conostoma*, therefore, the lenticular mass of tissue, which in the former descriptions (**27, 15**) is of conjectural history, is here interpreted as the remains of the tapetal layer. By reason of the thicker walls of its cells it did not deliquesce as did the internal tissue of the plinth in the formation of the pollen chamber, and after pollination was forced up through the plinth cavity into that of the lagenostome by a tent-pole development of the megagametophyte. In such manner the pollen grains would be effectively sealed in the pollen chamber.

Numerous pollen grains were found in the pollen chamber (figs. 2, 6). In one specimen ten were counted, and if they were uniformly distributed throughout the pollen chamber at least a third more must have been lost by the cut of the saw. The pollen grains are ellipsoidal, about 50 μ wide by 80 μ long. They have superficial reticulated markings and sometimes the walls appear to be wrinkled or slightly folded, but when seen in optical section they show no internal structure nor any indication of being multicellular (fig. 9). A unicellular condition of pollen grains would be at variance with the previous reports for *Conostoma*. Likewise, by analogy with modern gymnosperms, it would be contrary to the condition one would expect to find at this late stage in the ontogeny of the seed. Failure to find cells within the pollen grain might be attributed to lack of preservation, or it may be that the pollen grains are simply the exine remains after the formation and escape of spermatogenous cells.

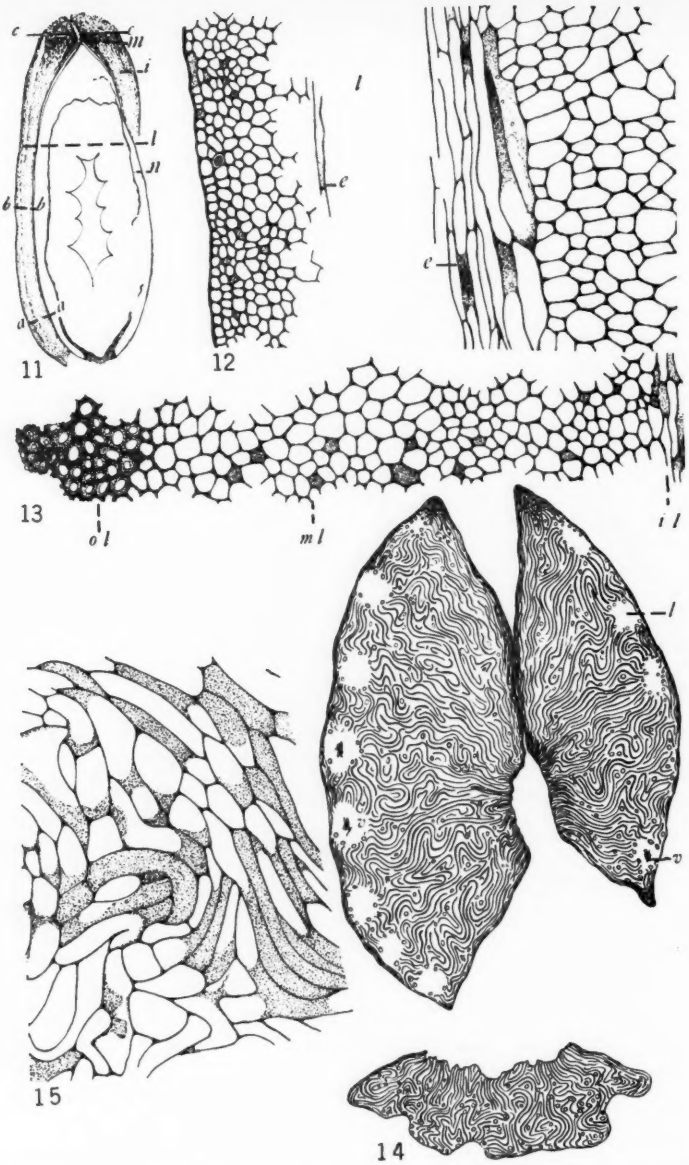
Pachytesta gigantea Ad. Br.

Two specimens of *Pachytesta*, referred to here as *Pachytesta* no. 1 and no. 2 respectively, were found by NOÉ at Polk's Patch near

Brownsville, Indiana. The coal here is also no. 5 of the Allegheny group of the Upper Pennsylvanian.

PACHYTESTA NO. 1.—This specimen has been figured under the name *Trigonocarpus* (7), which term was based upon the superficial character of the exposed portion of the seed and applied before its internal structure was known. When the coal ball containing the seed was sectioned, however, the previously labeled "seed" was revealed as a cast of the inner lining of the integument. It was also clear that the three ridges, which were largely responsible for the term *Trigonocarpus*, were not ribs of the integument as formerly thought, but mark the sutures or dehiscence slits of the integument. Since the integument has disappeared from the chalazal end the size of the seed can only be approximated, but a conservative estimate would make it about 7 cm. long by 3 cm. in transverse diameter. Exclusive of the integument, it measures 5.5 cm. long by 2 cm. in diameter.

A median longitudinal section through the chalazal end (fig. 11) indicates the relationship of the tissues in so far as they are preserved. The integument is thick and compact, hence the term *Pachytesta*. A detail through the integument shows three poorly defined layers (fig. 13). The outer layer, composed of isodiametric, relatively small, thick-walled cells, merges imperceptibly into the middle layer, the cells of which are distinguished from those of the former only by their slightly larger size and thinner walls. The middle layer in turn gives way to the inner layer, which is made up of several rows of thick-walled, elongated cells. While these elongated cells are the innermost ones remaining, yet it is not believed that they represent all the inner layer, but rather that they may have bordered thin-walled tissue, corresponding to the inner fleshy layer of modern gymnosperm seeds, which was not preserved probably because of the soft texture of the cell walls. At the micropylar end the integument is thicker, owing more to the character of the cells than to an increase in their number, for here they are larger in caliber, elongated, and sinuous (fig. 15). A transverse section at this level (fig. 14) shows the three valves of the integument, in the peripheral region of which are clear areas left by the disintegration of tissue. In some of these loculi are traces of organic material which



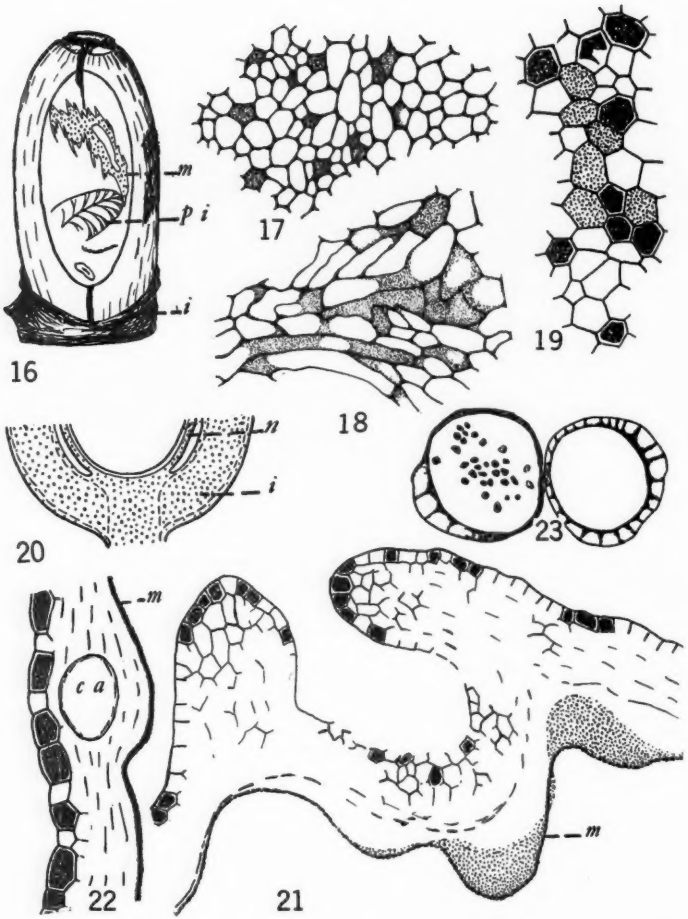
FIGS. 11–15.—*Pachytesta gigantea* no. 1. Fig. 11, diagrammatic representation of median longisection: *m*, micropyle; *i*, integument; *l*, loculus with indications of vascular tissue; *n*, nucellus. × about 1. Fig. 12, portion of longisection of integument at level of *b–b* of fig. 11, showing elongated cells, *e*, which border the loculus. × about 40. Fig. 13, portion of longisection of integument at level *a–a* of fig. 11 showing: *ol*, outer layer; *ml*, middle layer; *il*, inner layer. × about 40. Fig. 14, diagrammatic representation of transection of integument at level *c–c*: *v*, probably remains of vascular tissue. Fig. 15, detail of few cells of fig. 14. × about 40.

may well be remains of conducting elements, since it was from this region that BRONGNIART (**6**) and OLIVER (**25**) found and figured vascular strands. In longitudinal section these loculi are seen bordered by elongated cells which in texture and appearance simulate the elongated cells of the inner layer of the integument (figs. 12, 13).

In most of the seeds that have been described of the order Trigonocarpales (the seed order in which *Pachytesta* belongs) two vascular systems are recorded: an outer one which diverges from the main supply of the ovule at the chalaza and traverses the outer layer of the integument, and an inner system which is an extension of the chalazal bundles penetrating the nucellus to the floor of the pollen chamber. This latter system is described as consisting of a tracheal mantle as in *Stephanospermum* (**26**), or as being composed of discrete bundles as in *Trigonocarpus*. Since there were no recognizable vascular elements in this specimen of *Pachytesta*, it is hazardous to describe their possible position. Nevertheless, if the elongated cells of *il* figure 13 and *e* figure 12 are regarded as being of the nature of a bundle sheath, then the evidence indicates a double system confined to the integument: an outer system of twenty or more strands penetrating the outer layer of the integument and represented by the loculi (*l*) of figures 11 and 14 and an inner system of fewer strands all traces of which have disappeared except for the bordering elongated cells preserved as the inner limit of the integument.

The nucellus and adherent megaspore membrane are indistinct. This is not altogether because of poor preservation, for the nucellus has a shriveled appearance as if it had reached a membranaceous state before petrifaction occurred, as happens late in the ontogeny of modern gymnosperm seeds. It is therefore of some value in indicating an advanced stage in the development of the ovule, but the limited amount of preservation precludes any additional information in the nature of a contribution to the anatomy of the seed. There was no tissue preserved within the megaspore membrane.

PACHYTESTA NO. 2.—The integument had almost disappeared from this specimen except for some fragments near the chalazal end (fig. 16), but of these remaining fragments there was enough de-



FIGS. 16–23.—*Pachytesta gigantea* no. 2. Fig. 16, seed shown diagrammatically after removal of first section: *m*, megaspore membrane; *pi*, one of intruded pinnules; *i*, integument. About natural size. Figs. 17, 18, details of cellular structure of integument. × about 40. Fig. 19, surface view of epidermis of nucellus. × about 40. Fig. 20, diagram from median longisection of chalazal region showing divergence of integument, *i*, and nucellus, *n*. Figs. 21, 22, sections through nucellus and meagaspore membrane. × about 35. Fig. 23, two of sporangia, one containing spores, found in region of megagametophyte. × about 70.

tail of structure to establish relationship with the former specimen. This seed is slightly smaller than *Pachytesta* no. 1; exclusive of the integument it measures 4.8 cm. long by 2.2 cm. in transverse diameter.

The seed was cut at right angles to the plane of one of the ribs. Also median sections were secured through the chalazal and micropylar ends. Removal of the first section exposed the surface shown in figure 16. This surface would furnish almost a complete description of the seed as preserved, for the succeeding cuts supplied little additional information aside from making it possible to trace the course of the nucellus. Yet there are certain details of the tissues that are worth recording.

In the Trigonocarpales the nucellus and integument diverge in the chalazal end of the ovule (fig. 20). The nucellus consists of several layers of cells, the exact number not determined, of which the outer layer or epidermis is very conspicuous. A surface view of a group of these cells shows them to be roughly isodiametric, relatively thin-walled, and many of them filled with dark colored material (figs. 19, 21, 22). This last feature gives the layer so characteristic an appearance that the epidermis can be identified however fragmentary or disarranged it may be. Within the epidermis the amount of preservation is scanty. Only rarely is it possible to get the outline of a cell, but the few remaining ones do not appear to have collapsed and become membranaceous as in the former specimen. Such cavities as the one at *ca*, figure 22, are found in this tissue. They seem too regular to be the result of some chance irregularity of fossilization, and have the appearance of mucilage ducts. The preservation is so meager, however, that their nature is purely speculative. There is no trace of nucellar vascular tissue.

Within the nucellus and more or less adhering to it is the megaspore membrane, notable for its thickness. In modern seed plants the thickest megaspore membrane is found in the most primitive order, the Cycadales, where it may measure up to 10 μ thick, depending on the species and the stage of development. In *Pachytesta* the megaspore membrane attains a thickness of 15 to 20 μ. This exceedingly thick membrane might have been postulated for a group of plants at the level of the Cycadofilicales, the order of gymnosperms in which

Pachytesta belongs, since they must have evolved from a more primitive heterosporous condition in which the megaspores were shed earlier in their ontogeny and would need a thick membrane functioning as a protective layer.

These two layers, the nucellus and adherent megaspore membrane, are not found in their normal position following the contour of the integument; on the contrary they are much disarranged (fig. 16). This condition is not thought to have resulted from disorganization of tissues caused by germination of the embryo, rather it seems to be the result of some mechanical injury during development of the megagametophyte while the tissues were soft and pliable. The condition of the few remaining nucellar cells indicates that they were in a state of functional activity at the time petrifaction occurred. That there was some mechanical injury is further evidenced by the intruded rootlet, pinnules, sporangia, and other fragments of plant tissues (fig. 16). The absence of gametophyte or of embryonic tissue within the megaspore membrane would not therefore be accounted for by the germination and escape of the embryo.

Although proximity of plant structures, without actual organic connection, is no basis for establishing relationship, yet it is interesting and significant that the intruded pinnules, one of which is outlined in figure 16, have the recurved margins, the type of venation, and the structure of *Alethopteris*, a leaflet ascribed to *Medullosa*, the stem genus to which *Pachytesta* has been assigned.

Discussion

The foregoing descriptions but add to the mounting list of Paleozoic seeds or seed remains in which no embryonic tissues have been found. Theories, almost as numerous as the number of investigators, have been formulated for explaining the absence of embryos. None of them seem tenable, however, inasmuch as they presuppose certain given conditions; and while the given conditions may have existed and been responsible for the degree of preservation of the seed remains which suggested them, yet it would seem that these conditions or combination of conditions are fortuitous, and that it is possible and probable that Paleozoic seeds with embryos may be

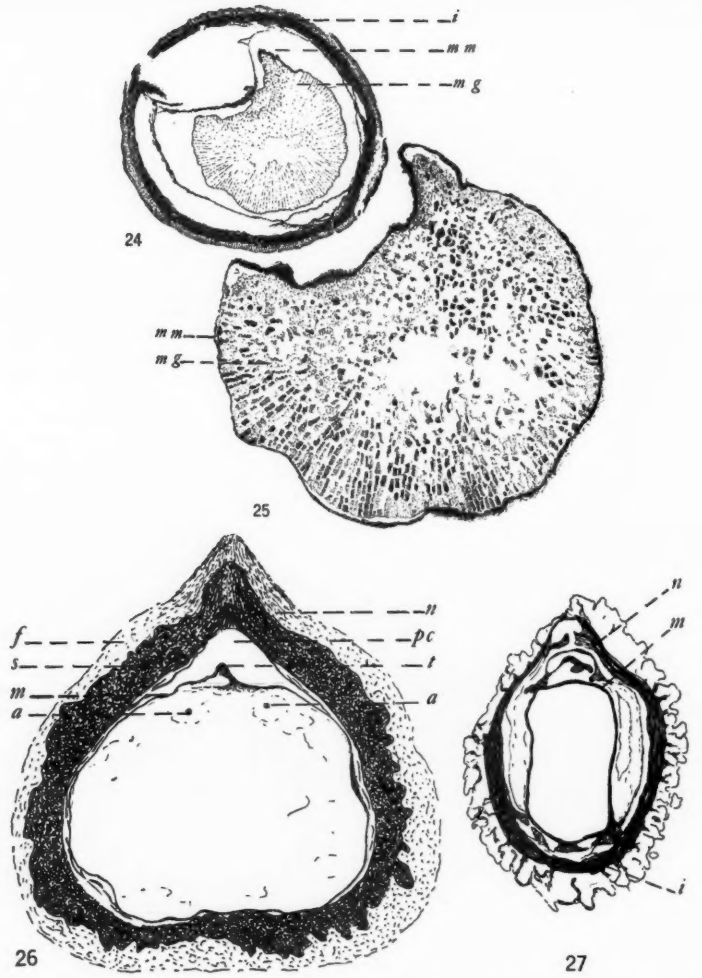
found.[1] That Paleozoic seeds are not peculiar or very different from their modern descendants in their method of development, and that there was variation in their age at the time of fossilization, is illustrated in the following series:

The transverse section of *Lagenostoma ovoides* (figs. 24, 25) has the resulting radial arrangement of cells of the endosperm or megagametophyte when the procedure of development is free nuclear division, partial placement, and subsequent centripetal wall formation. Without the median longitudinal section of this ovule one can only speculate on the reason for the sparsity of cells in the center. It may be that the process of wall formation had not progressed to completion; that wall formation ceased before segmentation was complete, as sometimes occurs in the ovules of modern gymnosperms; or that this tissue, lying in the path of an advancing embryo, was broken down and resorbed by it. This last condition could likewise find its counterpart in modern gymnosperms. It is to be regretted that there are no serial sections of this ovule available, for with such remarkable preservation, either archegonia or young embryos would surely be present.

While there is no endosperm preserved in *Pachytesta* no. 2, the condition of the nucellus suggests that the endosperm was in a developmental stage and had probably advanced to the formation of archegonia at the time petrifaction occurred.

The sharply differentiated and thick-walled tissues of the integument of *Conostoma oblongum* are characteristically mature. The presence of pollen grains in the nucellus not only establishes the incident of pollination, but their being devoid of contents indicates an ensuing period of development of microgametophyte. That there was a developmental period of the microgametophyte is supported by the condition of the nucellus, which, being the source of nutrition for the further development of the pollen grains after pollination, has here lost all of its internal tissue in the micropylar region as it would had it been absorbed by the functioning microspores. The proportionate size of the endosperm, as represented by its limiting layer the megaspore membrane, and the tent pole development indicated in

[1] Since the above was written, DARRAH has announced the discovery of *Cardiocarpon* embryos. Amer. Jour. Bot. **25**:98. 1938.



FIGS. 24–27.—*Lagenostoma ovoides*. Fig. 24, diagram of transection: *i*, integument; *mm*, megaspore membrane; *mg*, megagametophyte. × about 20. Fig. 25, detail of megagametophyte of fig. 24. × about 45. This section of *L. ovoides* (from British Coal Measures) kindly lent by Dr. G. R. WIELAND of Yale University, who has previously figured it (**33**). Fig. 26, *Cardiocarpon*, semidiagrammatic longisection: *f*, outer fleshy layer of integument; *s*, stony layer; *n*, nucellus; *pc*, pollen chamber; *t*, tent-pole development of megagametophyte; *m*, megaspore membrane; *a*, organic material occupying position of nuclei of fertilized eggs. × about 6.5. Fig. 27, *Trigonocarpon hookeri*, tangential longisection showing: *n*, nucellus; *m*, megaspore membrane; *i*, integument. This specimen, figured by DAWSON (**12**), lent by Redpath Museum of McGill University.

this material and demonstrated in the British specimens, are again evidence of a late stage in the history of the ovule. Yet the undisturbed appearance of the micropylar region of the nucellus, particularly the very small openings at both the base and apex of the lagenostome, makes it impossible to reconcile these specimens with "seed remains" after the germination and escape of the embryo.

Another seed significant in the developmental chain is that of *Cardiocarpon* (**28**). The preservation does not warrant a conclusive statement concerning the organic material at *a*, figure 26, but the position of these two bodies seems too regular to be chance. In its tent-pole prolongation and in its broad outline, the endosperm of *Cardiocarpon* presents a striking resemblance to that of *Ginkgo*. If one assumes a parallel development of the endosperm of the two genera, then the two dense bodies occupy the position of fertilized eggs. Were they the egg nuclei one would expect to find them toward the micropylar end of the central cell rather than centrally located as these appear to be.

The larger size of *Pachytesta* no. 1 and the membranaceous character of the nucellus indicate that this specimen may have proceeded to an embryonal stage; however, as in *Conostoma*, the integument and nucellus do not appear to have been disturbed by the emergence of a germinating embryo.

Any later stages of embryogeny that have been recorded are even more doubtful. DAWSON (**12**) writes of embryos and has one figured,—here reproduced in figure 27. The type specimen from which his drawing was made, together with some other seeds of the same collection, was kindly lent by the Redpath Museum of McGill University, with the permission to reinvestigate them. Figure 27 is labeled according to my interpretation. There seems no doubt that *m* is the megaspore membrane, and no doubt that there is no organic tissue within it. An examination of some of the other seeds of the collection showed them to be of similar preservation.

Summary

1. Structurally preserved seeds were found in coal ball material collected under the auspices of the Illinois State Geological Survey by Dr. A. C. Noé of the University of Chicago. The source of the

coal balls was from southern Illinois and Indiana in coal no. 5, which is in the Allegheny group of the Upper Pennsylvanian.

2. The seeds described are *Conostoma oblongum* Will. of the order Lagenostomales, and *Pachytesta gigantea* Br. of the order Trigonocarpales. This is the first description of *Pachytesta* from American coal balls.

3. Illustrations of *Lagenostoma ovoides* from the British Coal Measures, and of *Pachytesta gigantea*, *Conostoma oblongum*, *Cardiocarpon*, and *Trigonocarpon hookeri* from the American Coal Measures, are used to demonstrate similarity in development of Paleozoic seeds with those of modern gymnosperms, and to show variation in ontogeny at the time of petrifaction.

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TRANSPLANTATION EXPERIMENTS WITH PEAS. II

H. E. HAYWARD AND F. W. WENT

(WITH FOUR FIGURES)

Introduction

Previous experiments on the grafting of pea seedlings had led to the conclusion that: "Until actual union of the grafted peas through junction of the tissues by regenerated vascular bundles had occurred, no growth of the grafted tips, either of stems or leaves, took place" (4). Owing to the fact that many special cases were encountered in the grafting experiments which did not fit this conclusion, an anatomical study was made of the graft union to determine whether the type and rate of growth of scion were correlated with the anatomy of stock and scion, particularly with reference to the approximation of the original vascular tissues and the differentiation of new vascular connections. In addition, a number of grafting experiments were carried out to check certain details which had arisen as a result of the anatomical investigation.

The technique employed was that used by WENT (4). The axis of an etiolated seedling was cut obliquely through the median portion of the first internode, and the second internode was removed by making a similar cut through the third internode.[1] The two cut surfaces were then fitted together and held in place by a piece of glass tubing 5 mm. long, with a bore about equal to the diameter of the seedling axis. In early experiments, lack of good contact between the opposed surfaces of the graft was sometimes responsible for the failure of the graft to "take." The use of a device resembling a miniature miter-box to guide the razor blade eliminated this difficulty to a considerable degree, as the ends of the stock and scion could be cut at the same angle.

Alaska peas were germinated and grown in the physiological dark-room for one week; the graft was then prepared and daily length measurements of the entire axis were taken over a period of several

[1] The writers wish to express their thanks to Mr. G. C. WARNER for his assistance in the grafting experiments.

days. Seedlings representing each of the growth types were selected, the portions of the axes which included the graft were excised, killed, imbedded in paraffin, and serial longitudinal sections cut at 10 μ.

Investigation

Anatomy of first and third internodes

The structure of the lower internodes of the pea has been described by Hayward (1). Owing to the peculiar character of the transition zone, which includes the first three internodes, it is possible to distinguish between the first and third internodes in serial longisections. It is rather simple, therefore, not only to analyze the degree of vascular approximation of the original bundles and the amount of effective vascular tissue differentiated, but to determine the relative activity of the stock and scion.

In the first internode the vascular tissues of the stele consist of six groups of primary phloem, each of which is bounded on its outer face by a strand of phloem fibers and two distinct regions of primary xylem (fig. 1*A*). As seen in transection, the centrally located xylem consists of two crescentic strands whose convex surfaces are usually in contact at the center of the axis. There is some variation in this regard, and at the upper limits of the internode there may be a narrow band of parenchyma separating the two metaxylem groups. The four groups of protoxylem which form the peripheral portions of the central xylem strands are directed toward the two polar bundles of the stele, so that the relation of the protoxylem and metaxylem is exarch. The polar bundles which comprise the second region of the stele are endarch and collateral. There is a single-layered pericycle which is limited centrifugally by an endodermis with well defined Casparian thickenings. Cambium develops in the two polar bundles and in the zone of fundamental parenchyma between the centrally located metaxylem and the laterally placed phloem groups. This is relatively more active than the cambium of the root and may play a part in the formation of new tissues at the graft union (fig. 3*A*).

In the third internode the primary phloem groups and the polar bundles are oriented much like those of the first, but the laterally

oriented bands of xylem are widely separated by a central pith. The protoxylem and metaxylem are tangentially mesarch, with the protoxylem centrally located on the inner face of each band and flanked by groups of metaxylem elements (fig. 1*B*).

The cortical tissues are organized in an identical manner in the two internodes. There are two fiber bundles that lie in the same vertical plane as the polar bundles of the stele, and two fibrovascular

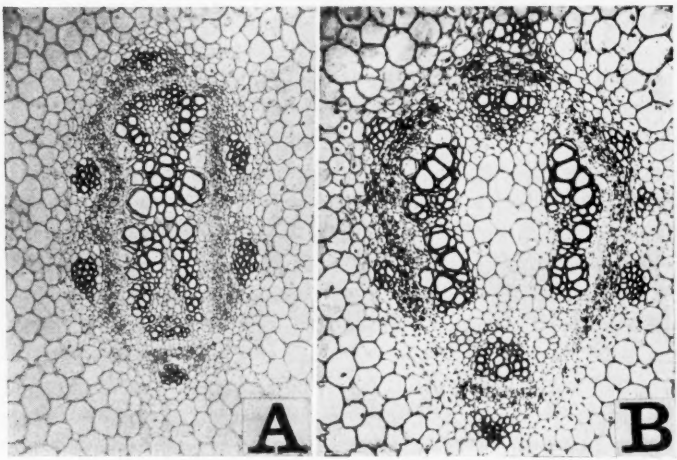

FIG. 1.—*A*, transection through stelar portion of first internode, showing arrangement of vascular tissues. *B*, same for third internode.

bundles that lie at right angles to them. In some cases large lacunae may develop in the parenchyma between the cortical bundles, but this was not observed in the young axes investigated in these experiments.

HISTOLOGY

The protoxylem consists of a limited number of slender annular and spiral elements which were probably nonfunctional at the time the graft was made. They appear much stretched as a result of axial elongation, the primary walls are collapsed, and in some cases the annular and spiral secondary wall thickenings are canted or pulled lengthwise. The vessel segments of the metaxylem are

reticulated or pitted, three to five times as long as broad, with transverse end walls. When present, the secondary xylem consists

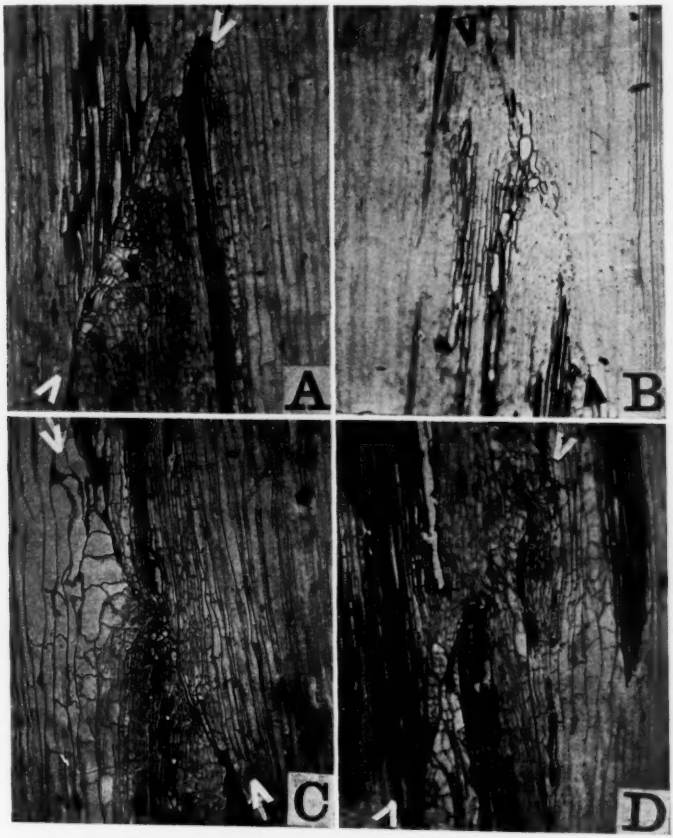

FIG. 2.—Longisections through graft unions. *A*, poor approximation of vascular elements of stock and scion and differentiation of new vascular tissue. *B*, same but with differentiation of new elements in scion. *C*, fair approximation of vascular elements with new xylem elements differentiating at junction of union. *D*, fair approximation of xylem elements and relatively good apposition of phloem. Scions are uppermost; arrows indicate plane of union.

of pitted vessels and xylem parenchyma. The vessel segments have greater diameter and less length than those of the metaxylem. In

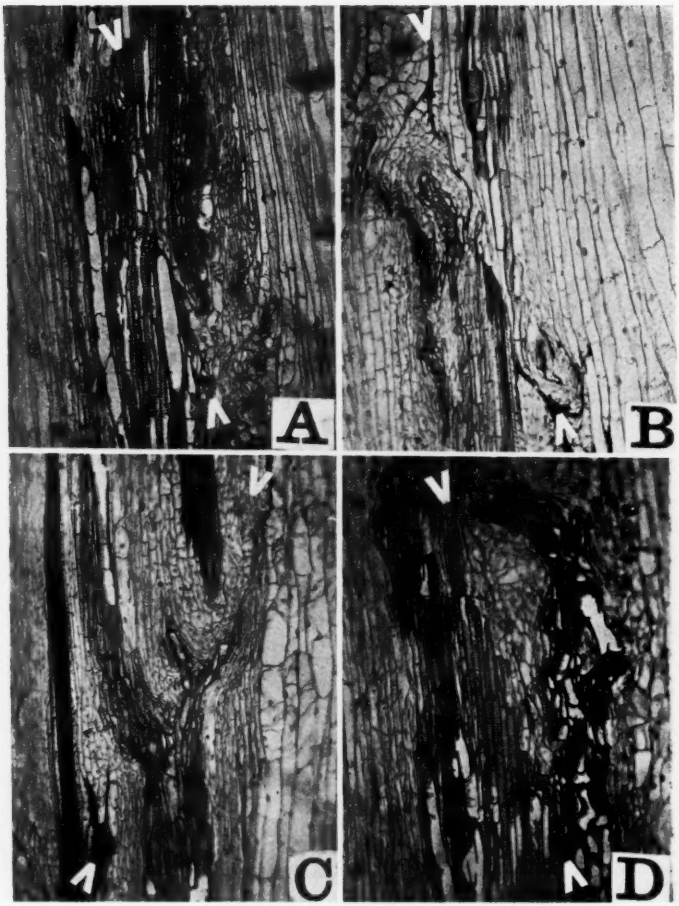

FIG. 3.—Longisections through graft unions. *A*, good approximation of xylem elements and accompanying cambial activity. *B*, fair approximation of xylem elements, formation of wound tracheids, and accumulation of deep-staining substances in vessels of stock. *C*, *D*, formation of new connecting vascular strands in cases where original approximation was fair (*C*) and poor (*D*). Scions are uppermost; arrows indicate plane of union.

the larger vessels there was frequently an accumulation of deeply staining substances which apparently plug up the lumina of the vessel segments for some distance from their cut ends (figs. 2*D* and 3*B*).

The wound tracheids which are formed in the parenchyma adjacent to original vascular tissue are irregular in shape and size, with reticulate or pitted walls. They are roughly isodiametric, or two to three times as long as broad. Those formed from cambium are laid down in rows and are more regular in shape, resembling the secondary vessels in this respect (fig. 3*A*).

The primary phloem consists of long slender sieve tubes and companion cells with adjacent strands of much elongated, thick-walled phloem fibers. There is relatively little primary phloem parenchyma. The cells of the wound phloem are usually irregular in size and shape, and in many cases resemble parenchymatous cells.

There is a definite spatial relationship between the new vascular tissues and the original vascular elements. In regions where the cut ends of xylem and phloem elements abut parenchyma, the activity of the latter is much accelerated, and it is at such loci that the majority of the wound tracheids and phloem elements are differentiated (fig. 2*A*, *B*). Although there is activation of the parenchyma on both sides of the graft, in general the parenchyma of the stock is much more active than that of the scion. In twenty observed grafts, meristematic activity and the differentiation of new vascular elements was greater in the stock in fourteen instances, and approximately equal in stock and scion in three other cases (fig. 2).

Anatomical analysis of graft types

The growth in length of 180 grafted peas was followed daily; and five and nine days after grafting, groups of peas representative of four different types of response were selected for anatomical investigation. In this series of grafts, a large number of grafted shoots started to grow rapidly immediately after grafting. In most experiments of this type this phenomenon is very rare, but fortunately it could be investigated in this case. Groups I to IV were collected five days after grafting; groups V to VII, nine days after grafting. Table 1 gives the mean growth in millimeters per day for each group.

A brief description of the anatomy of the graft in each group follows. It should be noted that in all seven groups, formation of new vascular tissue occurs to some extent. The differentiation of these tissues is usually initiated in the parenchyma abutting the cut ends of the vascular elements of the stock or scion, and in the parenchymatous regions adjacent to the vascular strands in either segment of the graft (fig. 2). In some instances the pericyclic tissue may be active in the formation of vascular elements; in others there was evidence of cambial activity (fig. 3*A*). In some of the types to be described, effective continuity of the vascular tissues of the stock and scion was not established during the growth period observed.

TABLE 1

MEAN DAILY GROWTH INCREMENTS FOR EACH GROUP (MM.)

GROUP	TYPE	FIRST DAY	SECOND DAY	THIRD DAY	FOURTH DAY	FIFTH DAY	SIXTH DAY	SEVENTH DAY	EIGHTH DAY	TOTAL
I.....	D	2.7	0.2	1.2	9.7	.....	.....	.....	.....	14.0
II.....	B	4.2	4.0	1.2	1.2	.....	.....	.....	.....	10.7
III.....	C	9.0	12.3	7.3	6.0	.....	.....	.....	.....	34.6
IV.....	A	10.0	17.2	5.2	2.5	.....	.....	.....	.....	35.0
V.....	A	10.5	12.5	10.5	6.0	5.0	2.0	3.0	0.0	40.5
VI.....	D	4.0	0.5	1.5	3.0	3.0	3.5	19.0	20.5	55.0
VII.....	C	7.5	9.0	7.0	12.0	10.5	27.5	44.5	21.0	139.0

GROUP I.—Growth response was characterized by little or no axial elongation during the first three days, followed by abrupt initiation of growth. This occurred in 10 per cent of all cases. The approximation of the original vascular elements of stock and scion was uniformly poor, with no direct juncture at any point in some instances. There were, however, effective vascular connections resulting from the differentiation of new xylem and phloem elements (fig. 2*B*).

GROUP II.—Growth response was characterized by little or relatively slow axial elongation, with gradual reduction in the daily rate and later complete cessation. Growth in length proceeded at the rate of a few millimeters a day, but after three days it had stopped completely. This occurred in 14 per cent of all cases. There was rather poor approximation of the original vascular elements of stock

and scion, and slight or no differentiation of effective new vascular tissue connecting stock and scion.

GROUP III.—Growth response was characterized by rapid initial axial elongation which continued at a relatively even rate. This occurred in 8 per cent of all cases. There was good approximation of the original vascular elements of stock and scion, and fair to good differentiation of effective new vascular tissue (fig. 2*C*).

GROUP IV.—Growth response was characterized by rapid initial axial elongation followed by slowing down or cessation of growth. Increase in length proceeded rapidly for the first two or three days, with subsequent reduction in the daily rate. This occurred in 68 per cent of all cases. There was good approximation of the original vascular elements, but very poor or no development of effective new vascular connections between stock and scion.

GROUP V.—Growth response was characterized by rapid initial axial elongation followed by cessation in the last three days. The growth period in this group was three days longer than in group IV. As in group IV, there was fair to good approximation of the original vascular elements, with poor or at best fair development of effective new vascular connections.

GROUP VI.—Growth response was characterized by slow initial growth and then rapid axial elongation. There was poor to fair approximation of the original vascular tissues, with good development of effective new vascular connections. Well defined bundles were differentiated which established continuity between stock and scion. This group is comparable with group I (fig. 3*D*).

GROUP VII.—Growth response was characterized by continuous axial elongation, the daily increments increasing during the final days of the observed period. There was very good approximation of the original vascular tissues as well as good development of effective new vascular connections similar to those found in group VI (fig. 3*A*).

Discussion

On the basis of the observed growth responses, the seven groups may be classified into two main categories representing four types. Groups II, IV, and V, which gave a common characteristic in the *ultimate cessation* of the growth rate, belong to types A and B.

Groups I, III, VI, and VII, which have a common characteristic in the *ultimate acceleration* of the growth rate, can be grouped as types C and D. These two ultimate effects can be correlated to some extent with the degree of approximation of the original bundles of the stock and scion, but are more directly correlated with the extent to which new vascular connections between stock and scion have been established by the differentiation of new vascular tissues.

Initial growth was limited in cases where the original bundles were not opposite (type B; group II), and fair to abundant where there was better approximation of these elements (type A; groups IV and V). In both types the ultimate slowing down or cessation of the growth rate can be correlated with failure to develop effective vascular continuity between stock and scion.

In types C and D a similar correlation exists. Initial growth was limited when the original bundles were poorly approximated (type D; groups I and VI), and was rapid when the apposition was good (type C; groups III and VII). In types C and D there was an ultimate increase in the growth rate, and in each there was good development of effective new vascular tissue. The degree of acceleration of the growth rate appeared to be in direct proportion to the amount of effective vascular tissue differentiated.

Another detail was observed, especially in types A and C. The original xylem elements were strongly infiltrated with a heavily staining material, more particularly near the cut surface (fig. 3*B*).

In attempting to correlate these data in a hypothesis, it becomes evident that it does not support the original contention of WENT (4) that there is "the necessity of cellular continuity between stock and graft to make the latter grow in length." In types A and C there is considerable growth the first three days after grafting. During this period no vascular continuity has been established, and in type A none ever develops. In exceptional cases growth may take place without vascular continuity, therefore, but in such instances there must be good approximation of xylem and phloem of stock to xylem and phloem of scion. Approximation of parenchymatous cells other than those of xylem and phloem alone is insufficient for rapid initial growth. This leads to the conclusion that the growth factors coming from the stock are moving through the vascular

bundles only. If it is assumed that the growth factors are transported, probably through the phloem, with a mass flow under pressure, as MÜNCH (2) supposes, then the observed facts are in harmony.

In exceptional cases there is such good approximation between phloem of stock and that of scion that the contents of the phloem of the stock may pass directly into that of the scion so that the latter can grow. Of course the greater part of the growth substances are lost by leakage along the cut surface. This is suggested in the first place by the reduced growth rate of the scion, which is at most one-third the original rate, and in the second place by the accumulation in the xylem of heavily staining extraneous material which must have been forced into the vessels. Whenever a complete new connection between bundles of stock and scion is established through the differentiation of one or more vascular strands across the cut surface, the flow of materials can follow this path without loss of pressure and growth at the original rate is resumed. A number of details, such as the apparent stopping of the flow a few days after grafting (in types A and C), could be explained with simple further assumptions, but they can better be postponed until more evidence has been collected.

One more fact should be pointed out. The conclusion that the grafts were more likely to take if they started to grow slowly (4) was borne out by this experiment. Of the grafts which grew rapidly the first days after grafting, only 10 per cent ultimately were successful. But of the slow growing grafts, 40 per cent succeeded.

The validity of some of the conclusions based on the foregoing anatomical evidence has been further tested in two additional experiments in which the grafts were made in such a way that better or poorer approximation of the bundles in stock and scion might be expected. The following types of grafts were made, all stems being cut through the third internode (fig. 4).

GROUP I.—Stems were cut and replaced in their original positions with as good approximation of the vascular bundles as possible. In one group (IA) the two ends were pressed together as firmly as the tissues would permit, and in another group (IB) the contact was so slight that the tissues just touched.

GROUP II.—Stems were cut obliquely and replaced on each other after a 90 per cent rotation of the scion. In this case the original bundles did not have very close approximation.

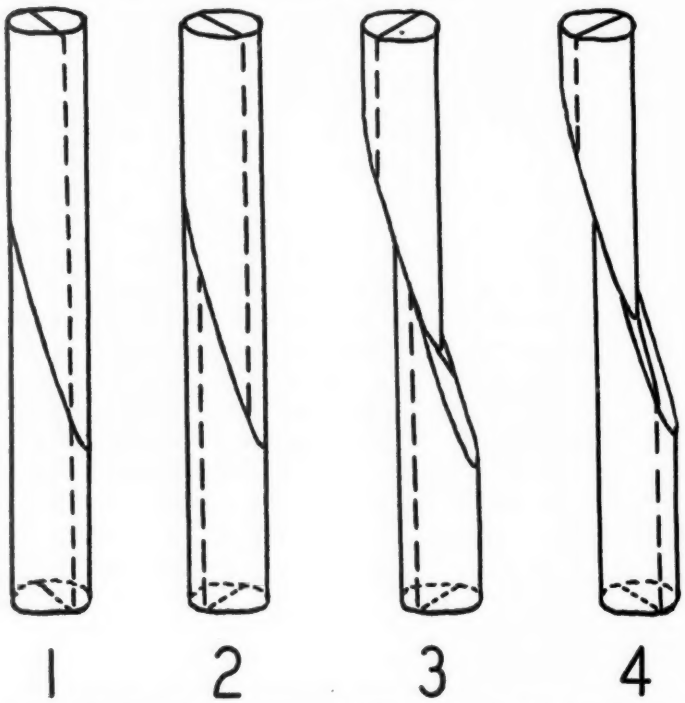

FIG. 4.—Diagrammatic representation of four main groups of grafts in experiments of table 2. Graft 1, stock and scion joined as cut; graft 2, after scion was rotated 90°; graft 3, after scion was slid halfway across cut surface of stock; graft 4, after additional rotation of scion. Dash line represents plane of the two cortical fiber bundles.

GROUP III.—Stems were cut as in group I, but the cut surfaces of the stock and scion were placed in such a position that there was no good approximation of the vascular bundles.

GROUP IV.—Stems were cut as in group III, but the scion was rotated 90 degrees on its axis and the cut surfaces were placed so that there was no approximation between the original vascular bundles of the stock and scion. Figure 4 diagrams the various types of grafts made. Table 2 gives the results of these experiments.

Growth of group IB was about the same as of IA, except that the maximal growth rate was reached somewhat later than in IA. In both experiments growth of group II was re-established rapidly. Group III was more or less intermediate. Group IV was slowest in starting to grow, having an extra lag of about two days as compared with groups I and II.

Despite these differences, ten days after grafting the same percentage of successful grafts finally took place in all groups and the

TABLE 2

Group	Period after grafting (days)											Maximum growth rate	Maximum growth after days	Grafts taking (%)	Ultimate length
	0–2	2–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13				
	Experiment no. 1														
Ia	0.7	0.6	0.7	1.7	6.7	15.0	16.2	18.2	19.6	21.0	20.5	39.0	7.5	67	455
Ib	0.6	0.5	1.2	2.4	4.0	7.0	11.0	15.5	17.2	17.0	17.0	37.5	9.5	65	396
II	0.8	0.6	1.6	3.4	10.1	15.6	17.6	19.3	23.4	24.1	25.1	38.4	7.5	80	436
III	0.8	0.6	0.3	2.1	6.4	9.7	11.5	15.5	22.8	19.3	19.0	39.5	8.5	70	440
IV	0.2	0.5	0.3	1.1	2.9	5.2	8.0	10.1	14.1	16.0	16.6	38.0	10.7	80	417
	Experiment no. 2														
I	1.6	3.0	3.4	13.2	17.4	19.5	13.6	13.0	12.4	11.7	11.0	36.4	7.5	67	362
II	1.6	2.0	3.6	19.2	21.4	21.5	18.2	13.4	9.0	6.8	4.6	37.8	7.3	75	359
IV	0.9	1.0	1.0	8.3	10.4	12.2	10.5	10.4	10.4	10.5	10.6	34.6	9.2	65	335

Mean growth rates in mm./day of all grafts of one group (including those which did not resume growth), each group consisting of 20–40 grafts. In the last columns are found the mean maximal growth rate of successful grafts, number of days after grafting this rate is reached, percentage of successful grafts, and mean length of successful grafts at end of experiment.

plants had the same maximal growth rate. This means that ultimately the graft union is equally well established in all four groups.

Only when the approximation of vascular bundles is slight, either as a result of mechanical contact (group IB) or topography (group IV, and to some extent group III), is there a somewhat greater lag between grafting and reaching the maximal growth rate.

Another interesting conclusion, corroborating one drawn from the anatomical investigation, can be reached. Initial growth for the first two day period is least in group IV; is greater but approximately the same in all other groups. This indicates that only with sufficient initial approximation of vascular elements of stock and scion can growth factors move into the scion, and that these move even before new vascular connections are established from the bundles of stock

to those of the scion. Therefore the movement of growth factors of the caline type seems to take place under pressure.

In an earlier paper (4) the conclusion was drawn that the overgrown cut surface, or the graft union, is no impediment to the translocation of food and growth factor, since the growth rate of the grafted shoots was approximately the same as for similar nongrafted shoots. A new experiment confirms this conclusion. A number of Alaska tops were grafted on Alaska stocks. One-half of the tops were grafted directly; that is, with only one graft union. In the other graft a piece of stem was interposed so that there were two graft unions. If the graft union was the factor limiting the growth of the shoot once the graft had taken, there would be a great difference between the two groups, the one with the two graft unions being much inferior to the single grafted peas. However, if the growth of the tops were determined by the base independent of the presence of the graft, both groups might be expected to exhibit equal growth. The results were as follows:

One graft union: Ultimately ten out of twenty grafts were successful and grew at a maximal rate of 26.5 mm. per day, this rate being reached eleven days after grafting.

Two graft unions: Ultimately fourteen out of twenty grafts were successful, and twelve days after grafting a maximal growth rate of 27.4 mm. per day was reached.

In the same experiments, some grafts of Daisy and Alaska tops on Daisy and Alaska bases were made. As in the earlier experiments (4) it was found that the growth rate of Daisy was exactly the same (9 mm. per day) whether grafted on Alaska or Daisy. And the growth rate of Alaska on Daisy was 27 mm. per day. These figures are reported to show that the preceding experiment was a representative one, although the maximal growth rate in all groups was somewhat slower than normal.

The conclusion is clear. Although the maximal growth rate is reached somewhat later in the double grafted stems than in the others, the ultimate growth rates are identical and the percentage of grafts that "take" is at least as good. Some double grafting experiments are now under way to determine what effect an intermediate

stem piece of a different variety may have on the stock-scion relationship (3).

Summary

1. The anatomy and histology of the graft union of etiolated pea stems have been investigated. Five and nine days after grafting, the graft unions were fixed and sectioned. Separation into four types was made between the grafts which grew rapidly or slowly to begin with and those which ultimately did or did not take. The following correlations were found:

2. In the grafts which grew rapidly in the first days, very good approximation between the original vascular bundles of stock and scion was found. When the approximation was poor, the grafts grew slowly to begin with. In the grafts which ultimately took, good development of new vascular connections between stock and scion was found. Such connections were almost or completely lacking in the unsuccessful grafts.

3. To test how far good or poor initial vascular approximation influenced the growth rate of the grafts, some special experiments were carried out which indicated that only the initial growth rate and the period before the scion reaches its maximal growth rate are affected by it. The ultimate maximal growth rate and the percentage of successful grafts were not significantly different in grafts with good or poor approximation. A result corroborating these conclusions was obtained when two graft unions were made instead of one. This also did not alter the maximal growth rate of the scion. Thus in peas the ultimate growth of the scion is not affected by the type of grafting or the graft union.

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COLORIMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF INDOLE(3)ACETIC ACID

JOHN W. MITCHELL[1] AND B. C. BRUNSTETTER[2]

(WITH THREE FIGURES)

Introduction

It has been found that cuttings from many kinds of plants respond, through increased root production, when treated with aqueous solutions of indoleacetic acid that range in concentrations between 0.01 and 0.2 mg. of acid per cubic centimeter (**2, 4, 6, 7**). To facilitate the study of the absorption of this acid by plants or plant parts from water solution, and to supplement available biological tests (**3, 8**), it is desirable to have an accurate chemical method with which aqueous solutions of the acid can be analyzed and any amount absorbed be determined by difference.

SALKOWSKI (**5**) reported a qualitative test in which a nitrite and a mineral acid were used to give a colored solution in the presence of indoleacetic acid (*Skatolecarbonsäure*) occurring among the products of protein putrefaction. He also found that a red color developed when ferric chloride was added to a solution containing this substance in the presence of a mineral acid. Other tests have been proposed but these two have been most commonly used in qualitative determinations of indoleacetic acid. WINKLER and PETERSEN (**9**) devised a quantitative test for indoleacetic acid, using as reagents copper, glyoxylic acid, and sulphuric acid. This method was also quantitative for the estimation of tryptophane. ZIMMERMAN and HITCHCOCK (**10**) recently applied WINKLER and PETERSEN'S test to extracts from plants that had previously been treated with indolebutyric acid. ALBAUM, KAISER, and NESTLER (**1**) recently modified the ferric chloride-hydrochloric acid test as a spot test technique and measured quantitatively the penetration of indoleacetic acid into *Nitella* cells.

In the present investigation the reaction of potassium nitrite

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(nitrous acid) and indoleacetic acid was studied in detail with respect to factors that affected the intensity and stability of the color produced. Other colorimetric reactions also studied, but in less detail, included (1) ferric chloride and indoleacetic acid; (2) nitrite and indolebutyric acid; and (3) nitrite and indolepropionic acid. A photometer employing a balanced photoelectric circuit was used. This instrument was equipped with a mercury lamp, the light from which was passed through appropriate filters. All measurements were conducted at a temperature of 24°.5–25°.5 C.

Experimental results

Nitrite test

In recommended tests for the qualitative determination of indoleacetic acid, concentrated nitric acid is added to a small volume of solution containing indoleacetic acid and potassium nitrite. A red color forms immediately, together with a precipitate which is perceptible in the case of solutions containing more than approximately 0.015 mg. of indoleacetic acid per cubic centimeter of solution. In general the photometric readings of any given solution increased for a period of time to a peak, following the addition of the reagents, and then slowly decreased. The addition of gum arabic as a protective colloid prevented the formation of the precipitate, but did not alter the general behavior of the reaction with respect to color formation. It was thus possible to control the reaction so that clear colored solutions were obtained, and difficulties concerned with the formation of a colloidal precipitate were avoided.

In order to improve the method further, the following factors were investigated individually: (1) amount and kind of acid; (2) amount of nitrite; (3) amount of indoleacetic acid present; and (4) effect of adding the mineral acid at different intervals of time following the addition of the other reagents. The effects of these factors on the intensity and stability of color were measured.

1. Acids.—Several mineral acids were used to adjust a known amount of indoleacetic acid solution containing potassium nitrite to a pH of approximately 0.9, and the percentage of light absorbed by different solutions was compared. Seventy-nine per cent absorption was obtained when the acidity of a solution containing 25 cc. of in-

doleacetic acid (0.05 mg. per cubic centimeter) and 0.3 cc. of 0.5 per cent potassium nitrite was adjusted with nitric acid. Under similar conditions, but using sulphuric and hydrochloric acids respectively, the percentage absorption was 78.4 and 80. When solutions were acidified to a pH of 2.5 with either acetic or nitric acid, approxi-

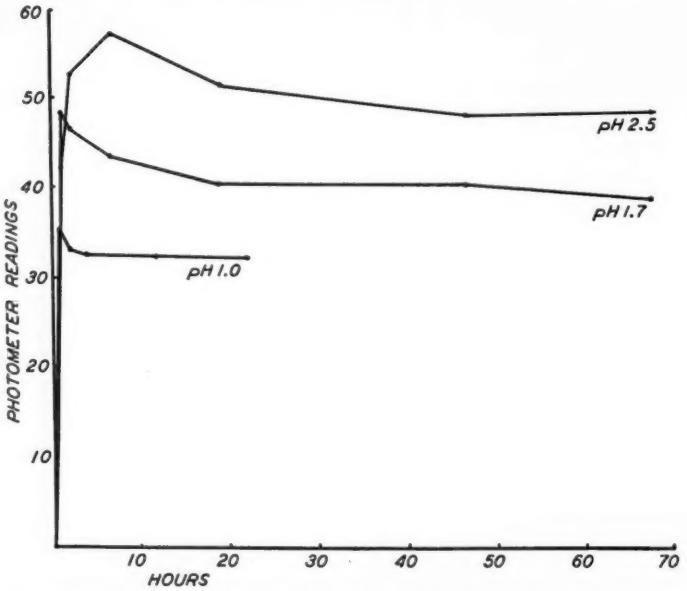

FIG. 1.—Effect of acidity on sensitivity and stability of color formed as result of reaction between indoleacetic acid and nitrite. Time expressed as hours following addition of reagents.

mately equal readings were recorded. Apparently the reaction between indoleacetic acid and nitrite was dependent on the pH of the reaction mixture and independent of the acid anion used, when the acid was added immediately following the addition of the other reagents.

The effect of pH on the time required to obtain equilibrium was studied by adding various amounts of concentrated nitric acid to 50 cc. of a solution containing 0.025 mg. of indoleacetic acid per cubic centimeter, 2 cc. of 0.5 per cent potassium nitrite, and 0.5 cc. of 5 per

cent gum arabic. Figure 1 shows that with increased acidity equilibrium was attained more rapidly than when less acid was used. The percentage absorption of light by solutions at a high acidity, however, was less than that of similar solutions that reacted at a lower acidity. Although increased acidity resulted in lower readings, it offered the advantage that less time was required for the attainment of equilibrium. Within the limits studied, pH had little effect on the stability of the solutions after equilibrium was attained. Maximum readings were obtained when the reaction occurred at a pH of ap-

TABLE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF NITRITE ON SENSITIVITY OF NITRITE TEST FOR INDOLEACETIC ACID. 0.625 MG. INDOLEACETIC ACID USED FOR EACH TEST AND PH OF EACH REACTION ADJUSTED TO 0.98. GUM ARABIC NOT USED

TEST	AMOUNT OF KNO_2 PRESENT (MG.)	MAXIMUM READING EXPRESSED AS SCALE DIVISIONS	TIME REQUIRED TO REACH MAXIMUM (MIN.)
1	0.25	87.4	10
2	0.5	101.0	5
3	1.0	107.0	4
4	2.0	99.5	4
5	3.0	86.0	4
6	4.0	74.0	3
7	5.0	61.1	2.5

proximately 2.5, a value well below that of the ionization constant of indoleacetic acid (1).

2. NITRITE.—The effect of nitrite concentration on color intensity was studied by adding various amounts of potassium nitrite to aliquots of solution containing a known amount of indoleacetic acid, and finally adding concentrated nitric acid. Preliminary experiments in which gum arabic was not used showed that the maximum color intensity attained depended upon the amount of nitrite present (table 1). The time required for the attainment of maximum color also depended to some extent upon the amount of nitrite used. Subsequent tests were carried out in which various amounts of 0.5 per cent potassium nitrite were added to 50 cc. aliquots of solution containing 0.5 cc. of 5 per cent gum arabic and 2500 mg. of indoleacetic

acid, and four-tenths of 1 cc. of concentrated nitric acid was finally added. The maximum intensity of color was produced when 0.1 cc. of nitrite was used, but the color was not stable under these conditions (table 2). It was therefore desirable to increase the amount of nitrite to 2 cc. and thus stabilize the system, although a lower intensity of color resulted.

3. INDOLEACETIC ACID.—To determine the relationship between the amount of indoleacetic acid present and the color intensity of the reaction mixture, 2 cc. of 0.5 per cent potassium nitrite, 0.5 cc. of 5

TABLE 2

EFFECT OF DIFFERENT AMOUNTS OF NITRITE ON SENSITIVITY OF NITRITE TEST FOR INDOLEACETIC ACID. GUM ARABIC USED

0.5% KNO_2 (cc.)	PHOTOMETRIC READINGS MADE AT DIFFERENT INTERVALS FOLLOWING ADDITION OF MINERAL ACID AND EXPRESSED AS SCALE DIVISIONS	
	3 HOURS	20 HOURS
0.05	69.1	58.0
0.1	109.3	99.7
0.5	101.2	88.1
1.0	86.5	82.5
2.0	60.0	61.7

per cent gum arabic, and 0.4 cc. of concentrated nitric acid were added to solutions containing different amounts of indoleacetic acid ranging in concentrations from 0.01 to 0.10 mg. per cubic centimeter. Immediately following addition of the reagents a red color developed in all the solutions. The initial intensity of this color decreased as the reactions came to equilibrium over a period of 2 hours, and in the case of the weaker concentrations, the color changed so that they appeared less red and more yellow, while that of the stronger concentrations remained red. Photometric readings, made with the aid of a filter that transmitted a wave length of approximately 546 mμ, bore a linear relationship to the amount of indoleacetic acid in the various solutions tested when measurements were made after equilibrium had been attained (fig. 2). The solutions were stable and the readings remained constant for 24 hours or longer.

4. TIME INTERVAL BETWEEN ADDITION OF NITRITE AND NITRIC ACID.—Gum arabic and potassium nitrite were added to aliquots of solution containing a known amount of indoleacetic acid. Nitric acid was then added after various intervals of time. A typical red color was developed immediately upon the addition of nitric acid irrespective of the time the indoleacetic acid and nitrite were allowed to react before the nitric acid was added. The color was more intense when the indoleacetic acid and nitrite were allowed to react for a

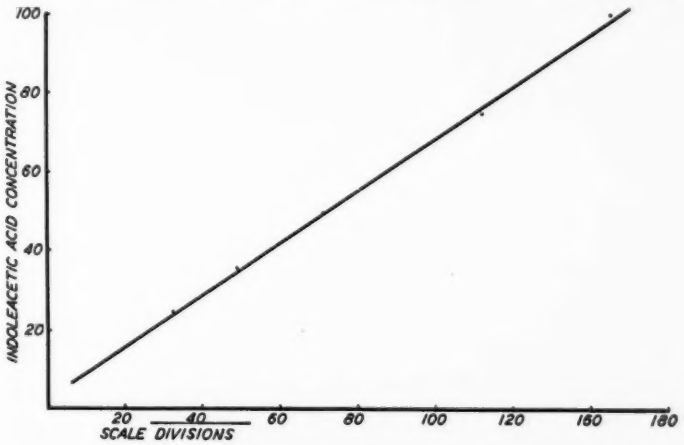

FIG. 2.—Effect of various concentrations of indoleacetic acid upon intensity of color formed as result of reaction of indoleacetic acid, potassium nitrite, and nitric acid. Concentrations of acid expressed as gamma (0.001 mg.) per cubic centimeter in solution tested.

period of time before nitric acid was added, than when it was added immediately (table 3). When indoleacetic acid and nitrite were allowed to react for 30 minutes or more, then added amounts of concentrated acid ranging between 0.1 and 2 cc. had no noticeable effect upon the intensity of color produced. The stability of color, however, was greatest when the acid was added immediately following the addition of nitrite. Stability was considered of greater importance than increased sensitivity and for this reason the acid was added, in subsequent experiments, immediately following the addition of nitrite.

SPECIFICITY OF NITRITE REACTION.—The nitrite reaction was tested for specificity by adding 2 cc. of 0.5 per cent potassium nitrite and 0.4 cc. of concentrated nitric acid to 50 cc. of solutions containing respectively: 0.05 mg. per cubic centimeter of (1) indoleacetic acid, (2) methyl ester of indolebutyric acid, (3) indolepropionic acid, (4) tryptophane, (5) indole, and (6) indolebutyric acid. The test gave a typical cherry red color only in the case of indoleacetic acid and indole, and a yellow color when indolebutyric acid, the methyl

TABLE 3

EFFECT OF TIME INTERVAL BETWEEN ADDITION OF NITRITE AND NITRIC ACID ON INTENSITY OF COLOR PRODUCED. GUM ARABIC AND POTASSIUM NITRITE ADDED TO 50 CC. ALIQUOTS OF SOLUTION CONTAINING KNOWN AMOUNT OF INDOLEACETIC ACID. NITRIC ACID THEN ADDED AT VARIOUS INTERVALS FOLLOWING ADDITION OF THE NITRITE

TIME BETWEEN ADDITION OF KNO_2 AND HNO_3 (MIN.)	23-HOUR READING EXPRESSED AS SCALE DIVISIONS
0	68.1
2	73.5
10	82.0
30	95.1
60	96.7

ester of indolebutyric, and the indolepropionic acids were used, while tryptophane gave no color.

To determine the applicability of the test to quantitative determinations of indolebutyric and indolepropionic acids, the reagents were added to various concentrations of these acids and the percentage of light absorption measured using a filter that transmitted a wave length of approximately 436 mμ. Although a detailed study of factors involved in these tests was not made, it is evident that the color intensity varied with respect to the concentrations used (fig. 3).

RECOMMENDED TEST FOR INDOLEACETIC ACID USING NITRITE

The following procedure is recommended for the estimation of indoleacetic acid present in aqueous solution in concentrations ranging from 0.01 to 0.2 mg. per cubic centimeter.

(1) 50 cc. of indoleacetic acid solution;
(2) Add 0.5 cc. of 5 per cent gum arabic;
(3) Add 2.0 cc. of 0.5 per cent potassium nitrite;
(4) Add 0.4 cc. of concentrated nitric acid immediately following addition of the nitrite.

FIG. 3.—Applicability of nitrite test for quantitative determination of indolepropionic and indolebutyric acids in water solutions. Photometric readings of solutions containing indolepropionic (broken curve) and indolebutyric (solid curve) plotted in terms of concentrations of acids expressed as gamma (0.001 mg.) per cubic centimeter of solution.

Shake and allow to stand at room temperature for 2 hours, after which time the color intensity will remain stable for 24 hours or longer.

The percentage of light absorbed by the solution can be measured with the aid of a photometer and compared with that absorbed by a

control solution containing a known amount of acid. With the electrophotometer used, cells 50 mm. in length and having a capacity of 25 cc. have been found convenient for this purpose. An alternative method of estimating color intensity consists of comparing the intensity of color produced through the use of unknown solutions with the intensity produced through the use of known concentrations of acid by means of Nessler tubes. The accuracy of this method has not been investigated.

TABLE 4

ACCURACY OF PROPOSED NITRITE METHOD FOR ESTIMATING INDOLEACETIC ACID IN WATER SOLUTIONS

DETERMINATIONS	CONCENTRATIONS OF INDOLEACETIC ACID		
	0.01 MG. PER CC.	0.025 MG. PER CC.	0.05 MG. PER CC.
1	4.2	21.0	63.7
2	4.0	21.5	62.9
3	3.4	21.2	63.7
4	4.3	21.4	62.9
5	4.3	21.4	64.6
6	4.4	21.5	64.5
7	4.1	21.3	64.0
8	4.3	21.3	63.7
9	4.4	21.5	64.5
10	4.3	21.3	63.7
Average	4.17	21.34	63.82
Standard deviation	0.0994	0.05213	0.2245
Coefficient of variance	2.38	0.24	0.35

The reproducibility of results obtained through the use of the nitrite method proposed was investigated by making replicate determinations on several solutions containing different amounts of indoleacetic acid and estimating the color intensity by means of a photometer. The coefficient of variability was less than 3 per cent for solutions containing as little as 0.01 mg. of acid per cubic centimeter (table 4). The effect of temperature on the results was found to be insignificant over a range from 15° to 30° C. During the use of the nitrite method it was noted that the 0.5 per cent potassium nitrite reagent employed remained stable over a period of 3 weeks, as indicated by frequent titrations.

The particular combination of reagents selected for the proposed method was one of several possible combinations. Other combinations, such as the use of less nitrite and nitric acid, and the addition of nitrite 30 minutes previous to the addition of the mineral acid, or the reduction in volume of all reagents in proportion, might prove advantageous in certain types of investigations when increased sensitivity is desired.

Ferric chloride reactions

A red color develops when a mineral acid is added to a solution containing indoleacetic acid in the presence of ferric chloride. A precipitate is not formed and the color varies somewhat with the concentration of mineral acid used. Two methods were found to be usable, one employing hydrochloric acid and iron in proportions similar to those described by Albaum, Kaiser, and Nestler (1), and the other, sulphuric acid together with ferric chloride. The former method, which was not studied in detail, was sensitive to concentrations of approximately 0.002 to 0.1 mg. of indoleacetic acid per cubic centimeter (table 5). The latter, which was studied in more detail, was extremely sensitive to small amounts of indoleacetic acid, the minimum total amount detectable being approximately 0.002 mg. A systematic study of the ferric chloride-sulphuric acid method was made concerning the effect of the following factors upon the stability and intensity of color produced: (1) concentration and kind of acid; (2) concentration of iron; (3) concentration of indoleacetic acid.

1. Acid.—A red colored solution was obtained when hydrochloric, phosphoric, nitric, and sulphuric acids were used. The time required for the color to reach a maximum intensity and its subsequent stability varied, depending to some extent upon the acid used. Sulphuric acid was found most suitable as its use resulted in a relatively stable color of high intensity. The ratio between the volume of acid and water present had a marked effect upon the sensitivity of the test, the maximum being attained when the ratio of the volume of acid to the volume of water present was approximately 1:1.5 (table 6).

2. Iron.—One-tenth molecular ferric chloride was used. Volumes of this reagent ranging from 0.01 to 10 cc. were employed to deter-

mine the effect of different amounts upon the sensitivity of the test. The use of relatively small volumes of iron solution decreased the sensitivity. On the other hand, the yellow color of ferric chloride

TABLE 5

EFFECT OF DIFFERENT AMOUNTS OF INDOLEACETIC ACID ON INTENSITY OF COLOR RESULTING FROM ADDITION OF FERRIC CHLORIDE AND HYDROCHLORIC ACID (ALBAUM, KAISER, AND NESTLER). TWENTY-FIVE CC. OF 3 PER CENT FERRIC CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID ADDED TO 25 CC. VOLUMES OF INDOLEACETIC ACID SOLUTIONS. REAGENTS PREVIOUSLY CHILLED TO PREVENT HEATING

INDOLEACETIC ACID PER CC. (MG.)	PHOTOMETRIC READINGS MADE AT DIFFERENT INTERVALS FOLLOWING ADDITION OF MINERAL ACID AND EXPRESSED AS SCALE DIVISIONS		
	2.5 HOURS	10 HOURS	24 HOURS
0.100	116.5	114.0	108.5
0.075	109.6	104.5	96.8
0.050	97.2	94.3	82.0
0.025	79.5	75.0	62.0
0.010	56.0	51.7	43.2
0.005	38.0	36.1	30.2
0.002	29.3	26.7	23.8

TABLE 6

SENSITIVITY OF FERRIC CHLORIDE-SULPHURIC ACID TEST AS AFFECTED BY DIFFERENT AMOUNTS OF WATER WITH RESPECT TO VOLUME OF MINERAL ACID PRESENT. MEASUREMENTS MADE 1 HOUR AFTER ADDITION OF ACID

INDOLEACETIC ACID PRESENT (MG.)	VOLUME OF WATER (CC.)	VOLUME OF H_2SO_4 (CC.)	PERCENTAGE LIGHT ABSORPTION
0.1	17.5	10	73.0
0.1	25.5	25	51.7
0.1	45.0	10	8.2

persisted when relatively large amounts of the reagent were used, and in this way interfered with the test by increasing the final color intensity. The volume of reagent required for maximum sensitivity was not sharply critical, however, being approximately 0.5 cc. for the method used.

3. INDOLEACETIC ACID.—Solutions containing various amounts of this acid were tested by adding 0.5 cc. of one-tenth molecular ferric chloride and 12 cc. of concentrated sulphuric acid to 18 cc. of solution containing different amounts of indoleacetic acid. Under these conditions photometric readings bear a quantitative relationship to

TABLE 7

EFFECT OF DIFFERENT AMOUNTS OF INDOLEACETIC ACID ON INTENSITY OF COLOR RESULTING FROM ADDITION OF FERRIC CHLORIDE AND SULPHURIC ACID. EIGHTEEN CC. OF SOLUTION CONTAINING DESIRED AMOUNT OF ACID AND 0.5 CC. OF M/10 FERRIC CHLORIDE ADDED TO 12 CC. OF CONCENTRATED SULPHURIC ACID

TOTAL AMOUNT INDOLEACETIC ACID GAMMA (0.001 MG.)	PHOTOMETRIC READINGS MADE AT DIFFERENT INTERVALS FOLLOWING ADDITION OF MINERAL ACID AND EXPRESSED AS SCALE DIVISIONS		
	1 HOUR	7 HOURS	14 HOURS
100	126.6	68.2	60.7
90	123.4	64.0	54.2
80	115.5	62.5	53.8
70	110.0	56.0	47.8
60	100.0	49.8	42.0
50	92.0	43.8	37.6
40	75.6	37.0	33.4
30	59.0	29.4	25.6
20	40.6	21.2	16.9
15	30.9	16.5	14.9
10	22.0	11.0	9.6
8	17.3	8.0	7.4
6	13.0	5.8	5.5
4	9.0	4.3	3.5
2	4.6	1.8	2.2

the amount of acid present, when total amounts of from 2 to 100 mg. of the acid are used (table 7).

The color resulting from the use of these reagents increased rapidly to a maximum and then decreased slowly in intensity. In the use of the method it is therefore necessary to take into account the time that elapses between the addition of reagents and the estimation of the color intensity. This difficulty can be compensated by including a series of known concentrations which should be treated the same as those containing an unknown amount of indoleacetic acid.

Recommended test using ferric chloride

The following method is recommended for the estimation of indoleacetic acid when present in total amounts of from 2 to 100 mg.

(1) Measure out 10 cc. of sulphuric acid (specific gravity 1.84) by means of a graduated cylinder;
(2) Add 0.5 cc. of M/10 ferric chloride;
(3) Add 14.5 cc. of distilled water and cool to room temperature;
(4) Add 5 cc. of aqueous solution of indoleacetic acid.

Shake and cool to room temperature before estimating color intensity by means of a photometer, Nessler tubes, or colorimeter.

Biological application

The methods of estimating indoleacetic acid as presented in this paper were investigated mainly for the purpose of developing a means of determining the amount of indoleacetic acid that cuttings, whole plants, or parts of plants absorb when placed in an aqueous solution of this substance. In preliminary experiments concerning the biological application of these methods, the amount of indoleacetic acid absorbed by geranium cuttings has been measured. Cuttings were made from plants grown under greenhouse conditions by cutting stem segments under water. Their cut ends were immediately submerged to a depth of 2 inches in a solution containing 0.05 mg. of indoleacetic acid per cubic centimeter. Similar cuttings in another group were partially wilted by allowing them to remain out of water for 1 hour before their cut ends were submerged in indoleacetic acid solution. The volume of water and amount of acid absorbed by the cuttings were determined after they had remained in the solution for 3 hours. Turgid cuttings cut under water absorbed more water and indoleacetic acid per unit of time than did those that were cut in air and allowed to become slightly wilted. It is evident that the nitrite test was applicable for measuring the amount of indoleacetic absorbed from water solution by the cuttings used.

It should be mentioned that some plants possibly contain substances that, when present in solution, would interfere with the quantitative tests proposed, and this factor should be considered as a possible source of error. The following test was conducted to de-

termine whether substances might diffuse from the cut ends of geranium cuttings or bean cuttings during treatment and thus interfere with subsequent tests made on the solutions to determine the amount of acid absorbed. The cut ends of the cuttings were submerged in 50 cc. of distilled water for a period of 3 hours. A known amount of indoleacetic acid was then added to a volume of the water in which the cuttings had been standing. The resulting solution was finally analyzed by means of the nitrite method described, and the amount of indoleacetic acid present was found to be equal to that previously added. It was evident that the test was unaffected by any substance that diffused from the cut ends of the plants.

Summary

1. Several colorimetric methods were studied to determine their applicability for the quantitative measurement of indoleacetic acid in water solution. These included the reaction between indoleacetic acid and (1) potassium nitrite and nitric acid; (2) ferric chloride and sulphuric acid; and (3) ferric chloride and hydrochloric acid. The nitrite and the ferric chloride-sulphuric acid tests were investigated in detail in order to determine the effect of the different factors involved upon the intensity and stability of the color produced.

2. The nitrite test proved to be applicable for the determination of indoleacetic acid in aqueous solutions having a range of concentrations from 0.01 to 0.15 mg. per cubic centimeter; the ferric chloride-sulphuric acid test was suitable for the estimation of total amounts of from 0.02 to 0.1 mg., under the conditions described. The ferric chloride-hydrochloric acid test was sensitive to essentially the same range of concentrations as that of the nitrite test, but was less suitable as the color produced was not stable.

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RELATION BETWEEN CHROMOSOME NUMBER AND STOMATA IN COFFEA

COARACY M. FRANCO

(WITH TWO FIGURES)

Introduction

RANDOLPH (5) showed the existence of a relationship between the number of chromosomes and the stomata in *Zea mays*, the tetraploid plants having fewer but larger stomata than the diploid ones. SAX and SAX (7), working with *Tradescantia*, also demonstrated the correlation between the chromosome number and the size and number of stomata. It was thought desirable to have similar information concerning the genus *Coffea*. The trees of this genus, part of the collection of the genetics department of this Institute, include a fairly complete polyploid series ($2n$ = 22, 33, 44, 55, 66, and 88 chromosomes), and were available for such an investigation.

Technique

METHOD OF COLLECTING.—After trying several methods for counting the stomata, the strip method was selected; that is, the epidermis is stripped off, mounted in a drop of water, and covered with a coverslip. Preliminary observations indicated that there is no appreciable difference in the number of stomata per unit area from different regions in the same leaf; nevertheless, the epidermis was always taken from the median part, midway between the apex and base and the midrib and margin. There is no difference in the number of stomata on the leaves at the tips of branches as compared with the numbers on leaves from the base of the branch, if the leaves observed are mature. The epidermis used, however, was always taken from leaves located at the third or fourth nodes, counting from the ends of the branches. Mature leaves were used in every case.

It was found that leaves taken from the tops of trees have a few more stomata per unit surface than those taken from the bottom; the difference is small but it was thought desirable always to take

the leaves from a point halfway up the tree. After detaching them, the leaves were carried to the laboratory with their petioles in water.

Since there appeared to be a larger variation in the number of stomata between the leaves of any two trees than between the leaves from the same tree, the mean was calculated from thirty leaves, taken if possible from thirty trees of the same variety. When only one tree was available, the thirty leaves were taken from it.

LLOYD'S method (3) was used for measuring the stomata: the epidermis was taken from the leaf and fixed at once in absolute alcohol. Here also the leaves were taken from the greatest possible number of trees and a mean calculated for each variety (or species). The material was collected between 8:30 and 9:30 A.M., when the stomata were open. NUTMAN (4) has shown that on clear days they may close before noon.

The counts were made with a reticulated 7× eye-piece and a 20× objective in a Zeiss microscope. This field area is equal to 0.3217 sq. mm. The epidermis covering the more pronounced ribs of the leaves was avoided because it does not have any stomata.

Measurements were made with a 6a Leitz objective and a 6× micrometric eye-piece. The length of the stoma was measured and its product multiplied by the constant 0.7854 to give the elliptic area of the opening; the results were converted into square microns.

CALCULATIONS.—In order to determine how many counts were needed to give a significant mean, sixty counts were made on *Coffea excelsa* because this particular species seemed to vary considerably with reference to stomatal number. The sixty counts were divided into two columns of thirty each, a mean calculated for each column, and the two means compared by FISHER'S (1) "*t* test." Thirty counts were sufficient to give a significant mean. The coefficient of variability was 17.96. In plants other than *C. excelsa* thirty counts were made and the coefficient of variability calculated; if the latter did not exceed 17.96, the mean was considered to be good.

The *bullata* variety, of the species *C. arabica* (tree no. 28, octoploid), gave a coefficient of variability of 20.90; however, computations similar to those worked out for *C. excelsa* revealed that here the mean based on thirty counts was statistically satisfactory.

All calculations were made for the number of stomata per field of

the microscope and this area converted into stomata per square millimeter. Twenty measurements were found sufficient to give a mean.

Since in some instances more data were needed for some species, and since only measurements made on epidermis collected within the same hour can be compared, it was thought desirable to collect forty leaves and to make measurements from these.

Data

Diploids

The diploid plants ($2n = 22$) showed the greatest number of stomata (haploid plants have not yet been discovered). Several species are known which have $2n$ equal to 22, and among these there is some variation in stomatal number; however, the variation is small compared with the differences found when diploids are compared with polyploids. The variation from species to species among the diploids called for consideration of them in groups. In the following members of the *C. excelsa* group (whose correct nomenclature may be questioned, as the synonymy of *Coffea* badly needs reworking), the species name is followed by the number of stomata per field, then by the number expressing the total of stomata per square millimeter.

C. excelsa group, $2n = 22$.—*C. abeokutae* Cramer 81.77, 254.14; *C. dewevrei* De Wild. et Th. Durand 75.23, 233.81; *C. dybowskii* Pierre ex De Wild. 80.93, 251.53; and *C. excelsa* A. Cheval. 69.70, 216.63. Total = 307.63, or a mean of 76.91 stomata per field. Mean for the number of stomata per square millimeter = 239.04.

C. robusta group, $2n = 22$.—*C. bukobensis* Zimmermann 107.00, 332.57; *C. quillou* Wester 124.10, 387.70; *C. laurentii* De Wild. 114.50, 355.87; *C. canephora* Pierre ex Froehner 102.33, 318.04; and *C. uganda* Cramer 97.20, 302.10. Mean number of stomata per field = 109.03; mean number of stomata per square millimeter = 338.86.

C. liberica group, $2n = 22$.—Only one tree available at the Institute. Average number of stomata per field = 104.87; average number per square millimeter = 325.93.

C. congensis group, $2n = 22$.—Only one plant available. Number

of stomata per field = 98.00; average number per square millimeter = 304.58.

Kawisari hybrid, $2n = 22$.—Highest number of stomata for *Coffea* occurs here. Average number of stomata per field = 139.17; number per square millimeter = 432.54.

TRIPLOIDS

Only two triploid plants available and these were synthesized by the genetics department of this Institute. Hybrid 45×37–1 showed 76.90 stomata per field and 239.00 per square millimeter, and hybrid 36×34–1 had 75.90 stomata per field and 235.90 per square millimeter. Average per field = 76.40; average per square millimeter = 237.45.

TETRAPLOIDS

Twenty-four specimens of *C. arabica* gave similar counts. Only one variety of this species, *monosperma*, was found to be exceptional. Its number is not included in the calculation of the mean. The data for these plants are presented in table 1.

PENTAPLOIDS

Only one pentaploid plant was available and it was too small to use.

HEXAPLOIDS

Two varieties of *C. arabica* were available, var. *bullata*, plant no. 133: 33.50, 104.12 and var. *bullata*, plant no. 270–21: 41.13, 127.83. Average stomata per field = 37.31; average number of stomata per square millimeter = 115.96.

OCTOPLOIDS

All are of the *bullata* variety of *C. arabica*. Only one tree showed the expected number of stomata (plant 28–54), and since this number did not agree with those found in the other octoploids it was omitted in calculating the mean. The data for these plants are: plant 28: 43.73, 135.91; plant 28–54: 23.20, 72.10; plant 56 (count made on mutated branches where $2n = 88$): 42.63, 132.49; and plant 210: 41.73, 129.70. The mean for all of these: stomata per square millimeter = 132.71; stomata per field = 42.70.

Table 2, summarizing the data presented so far, shows that an increase in the number of chromosome sets results in a decrease in the number of stomata on the leaves; however, mathematical proof of the correlation cannot be presented on account of the paucity of data.

TABLE 1

Variety of Coffea arabica	No. stomata per field	No. stomata per sq. mm.
typica Cramer (café nacional)	51.43	159.84
typica Cramer, flava form (café amarelo de Botucatú)	50.37	156.55
bourbon	55.27	171.78
bourbon, flava form (bourbon amarelo)	57.30	178.09
maragogipe	49.07	152.51
maragogipe, flava form (maragogipe amarelo)	46.67	148.16
nana*	61.30	190.52
angustifolia	56.03	174.89
bullata (plant 56)†	50.47	156.55
calycanthema*	53.00	164.72
cera*	53.97	167.74
columnaris	49.20	152.91
erecta	52.80	164.10
erecta, maragogipe form	52.43	162.95
goiaba	50.93	158.29
laurina	59.40	184.61
anomala*	47.70	148.25
mokka	62.83	195.27
monosperma	93.90	291.84
murta	57.93	180.05
pendula	53.33	165.75
polysperma	51.30	159.44
purpurascens	55.07	171.16
semperflorens*	59.60	185.24
Total = 1398.67		
M = 53.79		167.18

* Varieties not yet described.

† Part of this tree mutated to 88 chromosomes. Count recorded here was made from leaves on normal branches (where $2n = 44$).

The diploids have more stomata per surface unit than the triploids, the triploids more than the tetraploids, and so on. The correlation is well marked between the diploid and triploid species examined; however, *C. excelsa* has almost the same number of stomata as the triploid hybrids. This species has, among other characters, coarser leaves than the other diploid species. The average for the triploids was calculated from only two plants. These differed markedly in their appearance, but there is agreement as to stomatal number and the mean is to be taken as significant.

In the tetraploids ($2n=44$) the largest possible numbers were used in determining the mean. With the exception of the var. *monosperma*, all were very uniform with reference to size and number of stomata.

There is some confusion in the octoploids ($2n=88$). Their mean is higher than the one found for the hexaploids, but this difference did not prove to be significant statistically for the calculated $t=1.39$ as against the value given in the table (5.84 for $p=0.01$). Five trees of different genetic constitution were used. Plant 28–54 was the only one that gave the expected results, and as stated previously, this

TABLE 2

COFFEA	NO. OF CHROMOSOMES	MEAN
Robusta group	22	338.86
C. congensis	22	304.58
C. excelsa group	22	239.04
C. liberica	22	325.93
Kawisari hybrid	22	432.54
C. arabica×C. canephora	33	237.45
C. arabica	44	167.18
C. arabica	66	115.96
C. arabica	88	132.71

was not included in the calculation of the mean. In one tetraploid tree in which there had been a bud mutation, the octoploid leaves gave a different result from the tetraploid ones: for $2n=44$ the count was 156.86, whereas for $2n=88$ it was 132.49. The tetraploids gave the expected result but the octoploid gave a mean higher than was expected. The difference is statistically significant, for calculated $t=4.84$ as against t of the table, 2.57 where $p=0.01$. In spite of this it is clear that the number of stomata decreases as the number of chromosome sets increases.

It is interesting that the size of the leaves does not affect appreciably the number of stomata per square millimeter. For example, the varieties *nana* and *mokka* have a mean length of leaves of 1.10 and 5.0 cm. respectively, as compared with the varieties *maragogipe* and *typica*, with an average length of 14.22 and 12.20 cm. respectively. The width varies correspondingly. Although the

difference in leaf size is striking, the number of stomata per square millimeter is not outside the limits expected for the tetraploids.

The leaves taken from the apex of a tree have a few more stomata per unit surface than those taken from the bottom. This is in agreement with the findings of SALISBURY (6) and others.

Discussion

It was noted previously that the size of the stomata does not vary much between varieties of the same species having the same chromosome numbers; consequently, to ascertain whether or not the difference is a real one, a comparison was made between the varieties having the least and those having the greatest number of stomata. This lack of difference is shown by the fact that the means of given pairs of varieties (or species) are not really different on the basis of the t test. In each of the following three pairs the numbers, in the order given, represent the number of stomata per square millimeter; the area in microns; t calculated; and finally, t of the table, $p = 0.01$.

First pair, *C. arabica*, $2n = 44$.—Var. *mokka* 195.27, 22.84, 0.53, and 2.57; var. *anomala* 148.25, 25.16, 0.53, and 2.57.

Second pair, *C. excelsa group*, $2n = 22$.—*C. abeokutae* 254.14, 8.96, 1.62, and 2.57; *C. excelsa* 251.63, 11.46, 1.62, and 2.57.

Third pair, *robusta group*, $2n = 22$.—*C. quillou* 385.70, 12.04, 1.80, and 2.57; *C. uganda* 302.10, 9.47, 1.80, and 2.57.

The stomata of one tetraploid, one hexaploid, and one octoploid were measured, all three varieties belonging to the species *arabica*. Again the numbers given are for stomata per square millimeter; area in square microns; t calculated; and t of the table, $p = 0.01$. The chromosome number is indicated in parentheses after each name. Var. *cêra* (44): 167.74, 15.63, 1.56, and 2.57; var. *bullata*, plant 270–21 (66): 127.83, 19.25, 1.24, and 2.57; var. *bullata*, plant 210 (88): 129.70, 18.70, 1.24, and 2.57. From the value of t it is concluded that the size of the stomata does not vary between varieties of the same species having different chromosome numbers.

In order to determine whether the size of the stomata varies from species to species, one variety of each species with 22 chromosomes was measured. The numbers given after each name indicate the number of stomata per square millimeter and the size of the stomata

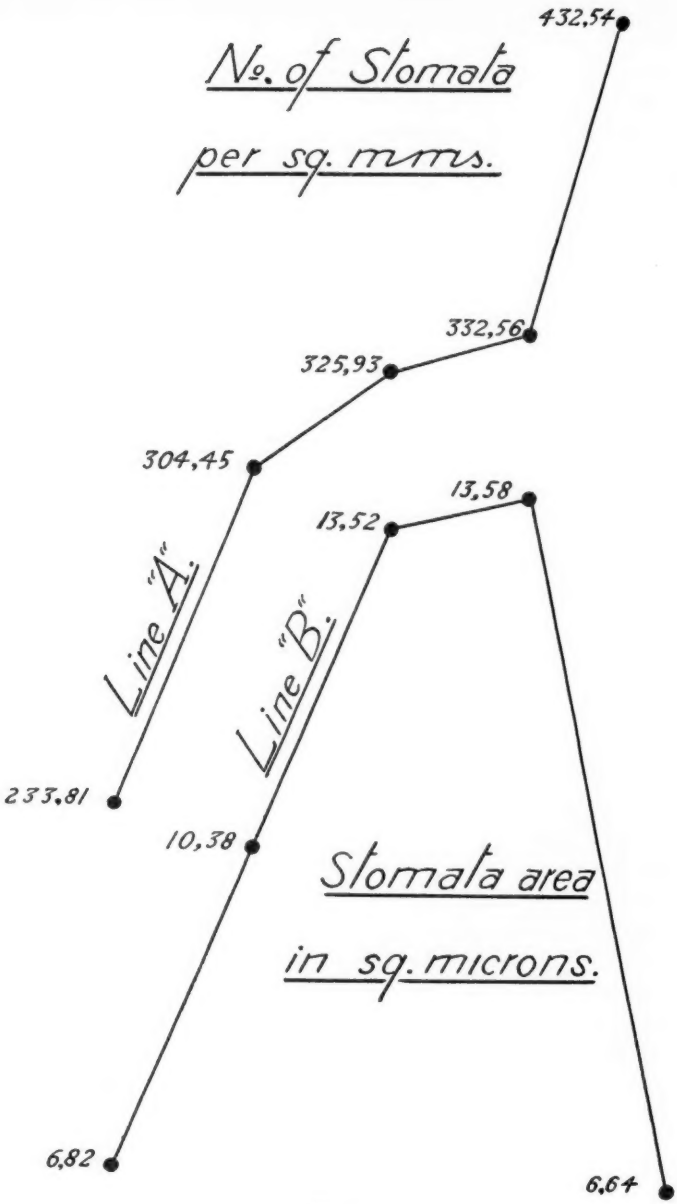
No. of Stomata
per sq. mms.
432,54
332,56
325,93
304,45
233,81
Line "A"
13,58
13,52
Line "B"
10,38
6,82
6,64
Stomata area
in sq. microns.

FIG. 1

100
200
300
400
No. of Stomata by mm. 2
No. of Chromosomes
88
66
44
33
22
C. arabica.
Var. bullata (n=22).
" " (n=22-24).
" " (n=25-26).
" " (n=11-12).
C. excelsa.
Var. bullata (n=33).
" " (n=22-23).
C. arabica.
Var. typica (café' nacional).
" flava form (café amarello de Bahia).
" bourbon.
" " flava form (bourbon amarello).
" maragogipe.
" " flava form (maragogipe amarello)
" nana.
" angustifolia.
" calycanthema.
" cera.
" columnaris.
" erecta bourbon.
" " maragogipe.
" goiaba.
" laurina.
" anomala.
" makka.
" monosperma.
" murta.
" pendula.
" polysperma.
" purpurascens.
" semperflorens.
plant n° 58.
Coffea liberica x canephora
hybrid 56 x 56-1.
" 45 x 33-1.
C. excelsa group.
Cabeadiba.
" stevensiae.
" dybowskii.
" excelsa.
robustoid group.
" buttobensis.
" kouillou. (C. guillon)
" laurentii.
" robusta.
" ugandæ.
C. congensis.
kawisari hybrid.
C. liberica

FIG. 2

in square microns. *C. dewevrei*, 233.81, 6.82; *C. bukobensis*, 332.56, 13.58; *C. liberica*, 325.93, 13.52; *C. congensis*, 304.58, 10.38; Kawisari hybrid, 432.54, 6.64.

Figure 1 summarizes the situation. Line *A* represents the data progressively from the smaller to the larger number of stomata in each species. Line *B* is plotted from the values based on the size of the stomata. It should be noted that the difference between the values 13.58 and 13.52 is not statistically significant, hence considering them as the same value the configuration of the curves would not be altered.

Figure 2 indicates that the size of the stomata varies from species to species. The hybrid variety, however, constitutes an exception when it is assumed that the size varies in the same way as the number. The exceptional type can be accounted for by the fact that it is a hybrid. HABERLANDT (2) found many abnormalities when he studied the stomata in forty-five F_1 hybrids. The stomata appear to be restricted to the lower epidermis; none were found in the upper epidermis in any instance.

In addition to the guard cells, the stomatal apparatus has subsidiary cells larger than the former. In general the subsidiary cells contain a large drop of oil which probably has some relation to the stomatal physiology. Green pigmented granules, like chloroplasts, are found in the cells of the lower epidermis in some varieties.

The writer concurs with NUTMAN'S statement that the stomata may close before noon on clear days; however, this does not occur if the tree is shaded.

Summary

1. The number of stomata in *Coffea* decreases as the number of chromosomes increases.

2. The size of the leaves has no influence on the number of stomata.

3. The area of the stomatal openings varies with the species and directly with the number of chromosomes.

4. The lower epidermis of several plants seems to have chloroplasts, especially the ones belonging to the group here designated as the *Coffea excelsa* group.

5. In general, the subsidiary cells contain a large drop of oil.

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EFFECT OF CALCIUM DEFICIENCY ON RESPIRATION OF ETIOLATED SEEDLINGS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 500

WENDELL R. MULLISON

Introduction

Few references in the literature are concerned with the effect of nutrient deficiency on respiration. SPOEHR and MCGEE (10) worked on the effect of amino acids supplied in nutrient solution to excised *Helianthus* leaves. HAMNER (2) studied the effect of nitrogen supply on the respiration rate of tomato and wheat. GREGORY and SEN (1) reported the effect of nitrogen and potassium on the respiration of excised barley leaves. JONES (3) worked on the respiration of etiolated wheat seedlings as affected by phosphorus. The present work deals with the respiration rate of tops and roots of etiolated plants in relation to calcium supply. Carbohydrate, protein, and fatty seeds were employed in this study.

Material and methods

Pea seeds of the Prince Edward variety, single cross hybrid selected corn obtained from Funk Brothers Seed Company, Bloomington, Illinois, and hubbard squash seeds were used. The weight of each seed varied at most not more than 15 mg. from that of the other seeds employed of the same kind. All seeds were sterilized in sodium hypochlorite, then soaked in distilled water for several hours until definite signs of swelling appeared. At that time they were placed in groups of ten in special beakers filled with sterile quartz sand previously thoroughly flushed with distilled water. Eight beakers were used for each determination. They were placed for germination in the darkroom in which the runs were made. Each day the plants of four beakers were supplied with a complete nutrient solution (7) and the other four with a minus calcium nutrient solution. This latter was the same as the complete nutrient solution except that calcium nitrate was replaced by potassium nitrate. Determinations of the respiratory rate were made when the seedlings were about a week old.

The temperature was kept as close as possible to 29° C., with a variation of only 1°. The darkroom was lighted by means of a 7 watt red bulb for only the brief time necessary each day to water the plants and to place them in readiness for the respiration determinations.

The apparatus which was built for this work was a modification of the one described in detail by MITCHELL (5), and was based upon the same principles. The amount of CO_2 given off by the plants was determined by the conductivity method. A CO_2-free stream of air was passed through a closed chamber in which the plants were growing. From here it was bubbled through humidifiers and then drawn through absorption towers. These towers contained 50 cc. of $\frac{1}{10}$ normal lithium hydroxide solution to which had been added 1 per cent butylic alcohol to lower the surface tension. The air flow was regulated to move at the rate of 20 liters per hour through the absorbent, the gases being dispersed as fine bubbles not more than 2 mm. in diameter. The towers were immersed in a water bath kept at a constant temperature that varied not more than 0.01° C. After passage through the absorption towers, the remaining gases flowed through copper tubing into a manifold where the negative pressure was controlled by a valve and indicated by a manometer. From the manifold, a tube led directly to an air pump which created the slight negative pressure required to draw air through the entire system. Sixteen sets of this apparatus were assembled as one unit and operated by a single air pump, accommodating the tops and roots of the eight sets of plants.

The towers were standardized separately by titration with $\frac{1}{10}$ N HCl, using phenolphthalein as the indicator. Thereafter the amount of CO_2 absorbed in the lithium hydroxide solution was determined by using a wheatstone bridge to measure the increased resistance of the solution.

Each chamber in which the determinations were made consisted of two parts, the lower part being the 1:5 liter beaker in which the plants were grown. It was provided with two outlets, one at the center of the bottom and the other one-fourth of the distance from the top. The upper part of the chamber was placed over the tops of the plants at the time of each run. It consisted of 4 inch pyrex tubing sealed at one end and with two side tubes for the incoming and

outgoing streams of air. In order to determine the respiratory rate of the roots as contrasted with that of the tops, they were separated from each other by a seal made of a mixture of paraffin, vaseline, and mineral oil that had a melting point of 40° C. The top part of the chamber was sealed to this paraffin mixture by a paste of clay and castor oil. The two compartments of the chamber were tested by means of a manometer to insure that they were air tight. Before the run the chambers were flushed with CO_2-free air for an hour. Each run involved approximately eighty plants and was of four hours' duration.

Investigation

Experiments with peas

Calcium deficiency became apparent immediately upon germination. The hypocotyls of the minus calcium seeds grew more slowly than those given the plus calcium solution, so that by the end of the fourth day the radicles of the latter were twice the length of the former. In the minus calcium plants at this time a cessation of growth of the primary root occurred, after which a few laterals grew feebly for a short time. Some of the minus calcium seeds, although producing roots, failed to push their epicotyls above the sand level. The calcium deficiency soon became so severe that necrosis appeared at the tips of the plants. Table 1 gives the data of a representative run.

TABLE 1

FOUR-HOUR DETERMINATION ON 7-DAY-OLD PEAS

CALCIUM	PART OF PLANT	CO_2 RESPIRED PER 80 PLANTS (MG.)	CO_2 RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops	60	3.5
	Roots	216	
Minus	Tops	32	2.4
	Roots	196	

Experiments with corn

Corn was also markedly sensitive to calcium deficiency. Here again the rate of growth of the minus calcium plants was slower than

that of the plus calcium. For the most part in the calcium deficient plants the leaves did not break through the coleoptile, while the plus calcium of the same age had two leaves unfurled. The gross appearance of the corn roots was not noticeably affected by the lack of calcium. Data are shown in table 2.

TABLE 2

FOUR-HOUR DETERMINATION ON 6-DAY-OLD CORN

CALCIUM	PART OF PLANT	CO_2 RESPIRED PER 80 PLANTS (MG.)	CO_2 RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops	148.8	6.96
	Roots	328.0	
Minus	Tops	79.2	5.27
	Roots	342.4	

EXPERIMENTS WITH SQUASH

Of the three plants used, squash was the slowest to show the effect of calcium deficiency. The rate of growth was retarded by lack of calcium, but necrosis did not set in at once. The length of the hypocotyl was affected while the respiratory rate was but little changed. Table 3 gives the results obtained.

TABLE 3

FOUR-HOUR DETERMINATION ON 6-DAY-OLD SQUASH

CALCIUM	PART OF PLANT	CO_2 RESPIRED PER 80 PLANTS (MG.)	CO_2 RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops	200	4.4
	Roots	152	
Minus	Tops	200	4.3
	Roots	146	

Discussion

The investigations which have been recorded regarding the function of calcium in plants indicate that it is concerned with permeability, toxic relationships, the actual formation of new cells, and the synthesis of protoplasm. TRUE (11) reported that calcium made

other nutritive elements physiologically available, this effect on permeability apparently being governed by calcium soaps and proteinates. Not only does calcium make other ions available, but in its complete absence plants undergo a leaching of material from their roots. Calcium also aids in preventing toxicity of other elements which in its absence prove harmful (**8**). NIGHTINGALE has described calcium deficiency symptoms in detail, noting that it first appears in meristematic regions.

With this understanding of the necessity of calcium to the plant, it follows that respiration in its absence would be modified. These results correspond with those obtained by other workers on the effects of nutrient deficiencies upon respiration. HAMNER (**2**) found that the respiration of tomato and wheat plants decreased when the nitrogen supply was limited. GREGORY and SEN (**1**) found that the effect of nitrogen and potassium deficiencies was to retard respiration. The latter work was done on excised barley leaves, and not on the entire plant; however, great care was taken to select only leaves of comparable maturity. They emphasize the fact that as individual leaves mature, their respiratory rate changes. JONES reported that the respiration rate of wheat seedlings increased upon the addition of phosphorus.

HAMNER first reported that the respiration rate of the roots and tops of any given plant or group of plants may be very different. Too often in considering respiration that of the tops alone is taken into account, thus neglecting root respiration which in tomato plants supplied with full nutrient solution and wheat seedlings is much more than that of the tops. In the work reported in the present paper it was found that the respiration rate of the roots in etiolated pea and corn seedlings was more than twice that of the tops. This is owing to the fact that there are many actively growing regions in fibrous root systems as contrasted with relatively few meristematic regions in the stem. Where there is less metabolic activity due to the lessened number of actively growing cells, one would expect a slower rate of respiration. In the squash seedlings this great difference in the rate of respiration of the roots as contrasted with that of the tops was not found. The respiration of the roots and tops was about the same, with the tops respiring slightly more. This was probably due to the fact that the cotyledons in

squash come above ground and are the source of food for the seedlings until the leaves are formed. Even so the respiration of the tops was not a great deal more than that of the roots. In corn and pea the cotyledons were not raised above ground, and therefore their respiration was included with that of the roots.

Of the three types of seeds (carbohydrate, protein, and fatty) of the same age, the seedlings which were of the high carbohydrate type respired most, the protein type respired least, and the fatty type was intermediate. Fatty seeds may absorb large quantities of oxygen in the formation of carbohydrates from fats during germination.

Although when roots and tops were separated, the roots of the minus calcium corn plants respired more than those of the plus calcium, the total respiration of the minus calcium plants was always less than that of the plus calcium. At the present time this increased respiration of the minus calcium corn roots presents an interesting unsolved problem. In this connection it may be noted that corn is a member of the grass family, which has been reported to require relatively little calcium (9). GREGORY and SEN reported a slightly increased rate of respiration due to a moderate potassium starvation, and only when the starvation became acute did the rate fall. If the roots do not suffer from calcium deficiency and are normal, however, their respiratory rate should be the same as the controls, whereas actually it exceeded them. In addition it is difficult to explain the severe minus calcium symptoms shown by the tops.

The effect of lack of calcium on squash is the least marked of the three types. Although minus calcium plants were much smaller in size, their respiration was approximately the same as those supplied with complete nutrients. There were few signs of necrosis in the minus calcium plants. Even though the plus calcium ones were larger, their respiration was but slightly more. It may be that the minus calcium squash plants had not yet begun to show a severe calcium deficiency, there having been enough available calcium stored in the seed to serve for immediate needs. This stored supply of available calcium probably varies with the type of seeds used, which may account for the difference in the response of the three kinds used in this work.

These results of calcium deficiency on respiration may be interpreted by considering lack of calcium a limiting factor in anabolic

plant processes; hence with fewer cells and less protoplasm, a lowered respiration rate must occur.[1]

A fact worthy of note was the effect on growth of the seedlings immediately after germination when supplied with the minus calcium nutrient solution. This caused a decided decrease in rate of development that was most pronounced in the case of pea. Pea seeds grew and formed radicles approximately 2 inches long, and small epicotyls, after which growth stopped and was not appreciably resumed. Whether this is due to the toxic action of other elements in the absence of calcium, which seems most likely, or to the effect on permeability or its lack in anabolism is not definitely known. Pea seeds contain calcium, but perhaps not much is available for germination. MAQUENNE and DEMOUSSY (**4**) reported finding a stimulating effect of calcium on pea seed germination. NIGHTINGALE (**6**) pointed out the extreme immobility of bound calcium in tomato plants. He also stressed the fact that when new cells are formed, calcium must be present, not only for the development of the middle lamella, but also for the synthesis of protoplasm. Since in seeds the amount available for use is small, much of the calcium necessary for the developing of new tissue from nearly the beginning must come from outside the seed. This factor, of course, would probably vary greatly with different types of plants.

Summary

1. The effect of calcium deficiency upon the rate of respiration of etiolated seedlings was studied. Seeds used in this study were pea, corn, and squash, examples respectively of the three general classes of seeds: protein, carbohydrate, and fat. The plants were divided into two parts, the tops and the roots, and the respiration of each was studied separately.

2. Total respiration under conditions of plus and minus calcium nutrition was highest in the corn seedlings, least in the pea seedlings, and intermediate in the squash.

3. Total respiration of the minus calcium plants was in every case less than that of the plus calcium plants.

[1] Work on calcium nutrition of the bean plant (GAUCH, H. G., Responses of the bean plant to calcium deficiency. In Press.), done subsequent to this investigation, shows that with plants given a minus calcium nutrient solution, the toxic effects are due to the other ions in the absence of calcium.

4. Lack of calcium was most noticeable in the greatly lowered respiration of the minus calcium tops as compared with that of the plus calcium tops.

5. Calcium deficiency lowered the respiration of the roots of pea and squash, but increased the root respiration of corn.

6. Roots of pea and corn respired more than twice as much as did their tops.

7. Respiration of the roots and tops of squash was about equal, although the tops respired more than the roots.

The writer is indebted to members of the department of botany of the University of Chicago for helpful suggestions made during the course of this study.

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VITAMIN B_1 IN RELATION TO MERISTEMATIC ACTIVITY OF ISOLATED PEA ROOTS

FREDRICK T. ADDICOTT

(WITH ELEVEN FIGURES)

Introduction

During the last five years the growth of excised roots has been studied intensively, particularly with regard to nutritional requirements. It has been shown by BONNER (1), and simultaneously and independently by ROBBINS and BARTLEY (7), that in addition to the nutrients of salts and sugar, vitamin B_1 is essential to the growth of excised roots. The vitamin affects not only the growth in length of the roots but also their general morphology. Roots grown with an excess of vitamin B_1 are so different in external appearance from those whose growth is limited by the available vitamin B_1 as to suggest the possibility of considerable difference in the internal structures as well. The present paper reports some effects of vitamin B_1 on excised pea roots grown in vitro.

Material and methods

The methods used were essentially those described by BONNER and ADDICOTT (3). Sterile technique was used throughout the handling of the cultures. Pea seeds (*Pisum sativum*) of the variety Perfection were sterilized to free them of mold spores and bacteria and were germinated in petri dishes at 25° C. At the end of two days, when the primary roots were about 1 cm. long, tips approximately 5 mm. long were removed by means of a surgical scalpel and placed in petri dishes containing 20 cc. of liquid culture medium. This medium contained mineral nutrients and sucrose, to which was added vitamin B_1 in certain cases (table 1). After the roots had grown in the medium for one week at 25°C., the tips were removed and transferred to fresh medium. These tips were always cut 1 cm. long so that the remaining basal portion of each root could be measured. This process of subculture was repeated weekly.

Added vitamin B_1 has no effect on the growth rate of the pea root

during its first week in culture, and consequently was always omitted from the medium during this period. As BONNER (2) has shown, the freshly excised pea root tip contains sufficient of the vitamin (and presumably of other growth factors) to maintain growth during the first week. However, the vitamin must be added to maintain growth in the later subcultures. Since roots to which the vitamin was not supplied ceased growth completely by the end of the third week, the material used in this investigation was not kept in culture longer than this time. At the end of each week, growth was measured and tips were fixed for cytological study. Roots from cultures which had

TABLE 1

BASIC MEDIUM FOR EXCISED PEA ROOTS

CONSTITUENT	CONCENTRATION (MG./LITER)
$Ca(NO_3)_2 \cdot 4H_2O$	236
$MgSO_4 \cdot 7H_2O$	36
KNO_3	81
KCl	65
KH_2PO_4	12
Fe-tartrate	1.5
Sucrose	4%
Vitamin B_1 (when indicated)	0.1 mg./liter

been maintained up to three weeks without added vitamin B_1 were compared with roots from cultures which had received additional vitamin B_1 during the second and third weeks.

Root tips were fixed in Navashin's solution. In order to eliminate any variation which might be due to diurnal fluctuation of the rate of mitosis, roots were always fixed at noon. Dehydration was accomplished by means of the tertiary butyl alcohol method and material imbedded in a rubber-paraffin mixture. Iron haematoxylin was used as the nuclear stain and fast green as the counter stain.

Investigation

The growth habit of excised roots is indicated by figures 1–3, which are taken from shadow photographs of roots at the end of the first, second, and third transfers. Roots after one week in culture show characteristically a swollen base, but at the end of succeeding transfers the root diameter is more uniform although there is a grad-

ual thickening toward the base. Figures 1–3 show also that the addition of vitamin B_1 to the culture medium results in sustained growth in length of the root. Roots supplied with the vitamin are smooth and white with a slightly yellowish meristematic region when observed at the end of a transfer period. The roots not supplied with vitamin B_1 are short, brownish, and irregularly swollen.

Sections of root tips of seedlings grown for one week in garden soil in the greenhouse were used as a basis for comparison with the sections of excised roots. Roots at the end of the first week in culture

FIGS. 1–3.—Shadow photographs of excised roots, taken at end of each weekly transfer. Fig. 1, end of first week, not supplied vitamin B_1. Fig. 2, end of second week, supplied vitamin B_1, above; not supplied, below. Fig. 3, end of third week, supplied vitamin B_1, above; not supplied, below. $\times \frac{1}{2}$.

and from later transfers which had received vitamin B_1 appeared in most respects to be closely similar to the seedling roots. They were thinner in diameter, a difference brought about primarily by a decrease in the number of axial rows of cells in the excised roots. Table 2 gives the results of counts of the number of cells along a diameter of a transverse section through the region of elongation. This is equivalent to the number of axial rows of cells which would be found in a median longitudinal section of the root. This table shows that as the roots grow in length during the first three weeks fewer rows of cells arise in the meristem. In cultures to which vitamin B_1 has not been added, and which have correspondingly grown but little in length, this effect is not marked.

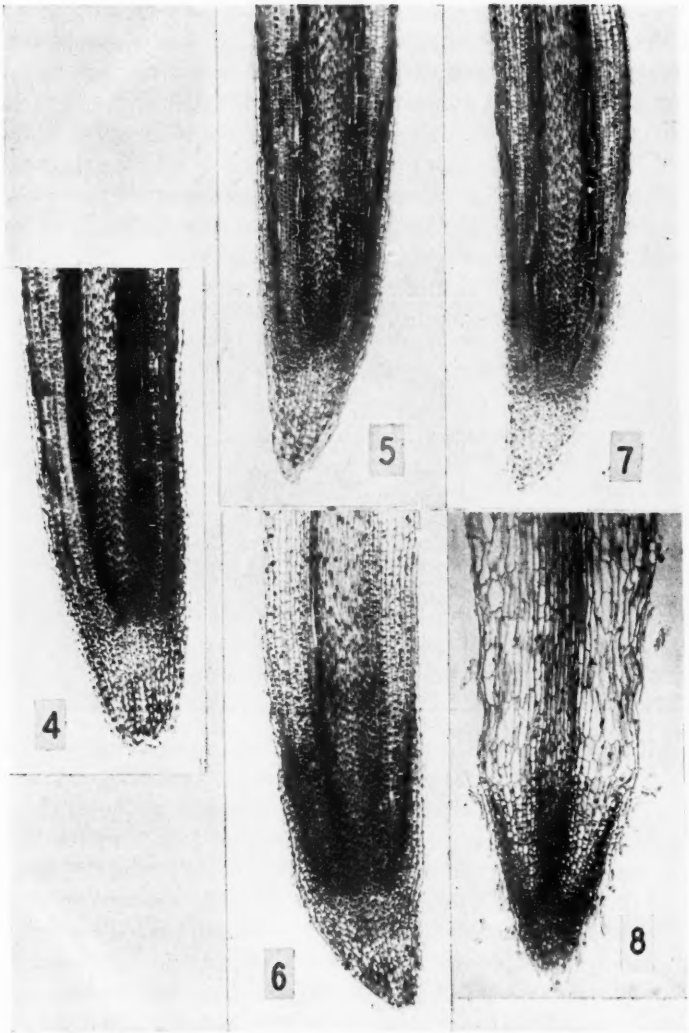
The most striking microscopic differences between roots grown with and without the addition of vitamin B_1 are those to be observed in the meristematic region. Roots supplied with vitamin B_1 are essentially normal and possess an extensive and active meristem at the end of the third week. In roots which have not been supplied vitamin B_1 the meristem shows a marked reduction in both extent and activity after three weeks, and a large proportion of the cells which were originally densely filled with cytoplasm now are highly vacuolated. These differences can readily be seen in the photomicrographs of this region which illustrate the effect of vitamin B_1 on the meristem of the roots (figs. 4–10).

TABLE 2

NUMBER OF AXIAL ROWS OF CELLS IN PEA ROOTS GROWN UNDER VARIOUS CONDITIONS

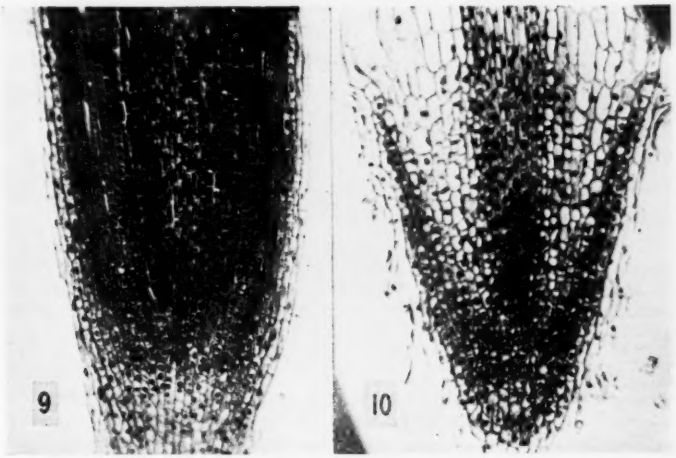
SOURCE OF ROOT TIP	TIME OF COUNT	NUMBER OF CELLS ON DIAMETER OF TRANSVERSE SECTION OF ROOT	
		NOT SUPPLIED B_1	SUPPLIED B_1
Seedlings:			
Primary roots	End of first week	48.5 ± 2.8	.....
Secondary roots	End of first week	39.8 ± 0.96	.....
Excised roots	End of first week	41.1 ± 0.47	.....
Excised roots	End of second week	42.0 ± 2.2	39.5 ± 1.0
Excised roots	End of third week	39.4 ± 1.4	36.3 ± 0.57

Although vitamin B_1 exerts a marked effect on meristematic activity, roots deficient in the vitamin are nevertheless unaffected in certain other respects. Cell elongation and maturation of the cells arising from the meristem continue to completion. Since very few new cells are formed after the roots become deficient in vitamin B_1, nearly all the cells of these tips are mature. The walls of mature cells are well developed in roots cultured for three weeks without the addition of vitamin B_1. Annular and spiral thickenings of the xylem vessels are laid down, and have frequently been observed within a distance of a few cells from the short meristematic region. The unusual development of the vacuoles should also be mentioned, since this process has attracted much attention; for example, GUILLIERMOND (5). After roots have grown for three weeks without the addition of vita-



FIGS. 4–8.—Longisections of root tips taken at end of each week in culture. Fig. 4, end of first week, not supplied vitamin B_1. Fig. 5, end of second week, supplied vitamin B_1. Fig. 6, end of second week, not supplied vitamin B_1. Fig. 7, end of third week, supplied vitamin B_1. Fig. 8, end of third week, not supplied vitamin B_1. $\times 59$.

min B_1 there are large vacuoles in the cells of the meristem in the region from which the cortical elements are derived. In these same meristems, the cells from which stelar elements develop appear more like those in the seedling roots, although these cells are greatly reduced in numbers. The vacuoles remain small in all the cells of the meristem when vitamin B_1 is added to the culture medium. That the cells of roots deficient in vitamin B_1 are metabolically active is also shown by the fact that they store starch abundantly. The deposition



FIGS. 9, 10.—Root tips at end of third week in culture. Fig. 9, apical portion of meristematic region of root supplied vitamin B_1. Fig. 10, meristematic region not supplied vitamin B_1. ×114.

is greatest in the more proximal regions of the roots, but starch grains, like the vacuoles, are conspicuously present even in cells adjacent to the meristem. These points can be seen to a limited extent in figures 4–10.

In order to obtain a quantitative relation between growth rate and meristematic activity of roots in culture, counts were made of the number of mitotic figures to be found in the meristem of median longitudinal sections of roots subjected to the different treatments. This count is a function of the mitotic frequency. The length of the apical meristem was also measured. These results, together with the growth rates of the corresponding roots, are summarized in ta-

ble 3. They are also presented graphically in figure 11, where for ease of comparison the growth rate, the number of cell divisions, and the length of the meristematic region of the roots at the end of the first transfer are taken as 100 per cent in each case.

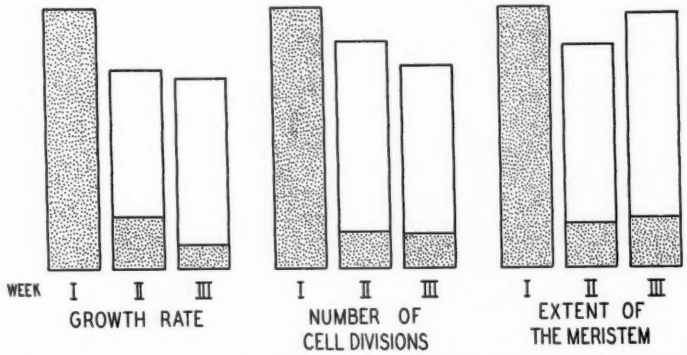


FIG. 11.—Diagram comparing growth rate, number of cell divisions, and extent of meristem of root tips studied, from data of table 3. In each case the value for the first week was taken as 100 per cent. Stippled areas correspond with roots not supplied vitamin B_1 and are superimposed upon clear areas representing roots supplied vitamin B_1. No roots supplied vitamin B_1 during the first week.

TABLE 3

COMPARISON OF GROWTH RATE, CELL DIVISIONS, AND EXTENT OF MERISTEMATIC REGION IN EXCISED PEA ROOTS

	First week	Second week		Third week	
	Minus vitamin B_1	Minus B_1	Plus B_1	Minus B_1	Plus B_1
Growth rate (mm./week).....	59.2	12.0	45.6	5.6	43.5
No. of cell divisions*.....	18.0	2.5	15.7	2.4	14.0
Extent of meristem (mm.)†....	1.55	0.27	1.33	0.30	1.52

* Because of the difficulty of identifying with certainty the early prophase and late telophase stages, only metaphase and anaphase stages were counted. Figures are a measure of the number of cell divisions per median longitudinal section.

† These figures indicate linear extent of apical meristematic activity in root tip. Distance measured was from tip of meristem to last observable mitotic figure which was definitely a part of the apical meristem.

It is now well established that vitamin B_1 may be regarded as a hormone of root growth. It has been shown (4) that vitamin B_1 is produced by leaves in the light and is transported to the roots, where

it affects growth in length. From the preceding observations it is evident that the effect on root growth is in part (and perhaps largely) attributable to an effect on meristematic activity. It will be of interest to determine whether the effect of vitamin B_1 on meristematic activity is in turn attributable to the co-carboxylase function of the vitamin (**6**).

The effects of vitamin B_1 are thus quite different from those of the auxins which primarily affect cell elongation. With isolated roots there is a close positive correlation between the supply of vitamin B_1 and growth rate, number of cell divisions, and extent of the apical meristem. The effect of vitamin B_1 as a hormone of root growth is thus correlated with the maintenance of normal meristematic activity.

Summary

1. The action of vitamin B_1 as a growth hormone of roots is through an effect on meristematic activity rather than on cell elongation, which is the primary effect of the auxins.

2. Cell elongation, differentiation, and maturation proceeded normally in the roots to which vitamin B_1 was not supplied, as far as could be observed, even though meristematic activity was greatly reduced.

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VITAMINS AND THE GERMINATION OF POLLEN GRAINS AND FUNGUS SPORES

WILLIAM C. COOPER

(WITH TWO FIGURES)

Introduction

Various workers have shown that the addition of extracts of fresh plant tissues of various sorts induces the germination of pollen grains and fungus spores. KNOWLTON (4) observed that the percentage germination of *Antirrhinum* pollen was greatly increased by the addition of minute amounts of crushed stigma. WILCOXON and MCCALLAN (10) found that the addition of small quantities of tomato, orange, apple, or pear juice and aqueous extracts of potato tubers resulted in nearly 100 per cent germination of spores of *Penicillium*, *Glomerella*, *Botrytis*, and *Sclerotinia*. A water extract of sterilized yeast was likewise found to induce germination of pollen (2) and fungus spores (10).

It is now generally known that vitamin B_1, vitamin B_2, vitamin B_6, nicotinic acid, and a complement of amino acids are among the constituents of yeast extract. And of greater interest are the recent findings of BONNER (1) and ROBBINS and BARTLEY (5) that the beneficial effect of yeast extract on growth of excised roots in vitro is because of its vitamin B_1 and amino acid content. It would therefore seem logical to expect that the stimulating effect of crude extracts on pollen and fungus spore germination might be attributed at least in part to their vitamin content. It was with the hope of identifying known chemical substances, such as the vitamins, concerned with pollen and fungus spore germination that the present work was undertaken.

The pollen used in this investigation was obtained from four horticultural varieties of *Carica papaya* which were inbred by sib-pollination for a number of years by TRAUB and ROBINSON (9). The spores used consisted of *Colletotrichum gloeosporioides* from the avocado and papaya, and *Penicillium italicum* from the orange.

Water solutions of several of the vitamins and a number of other

substances were tested individually for effect on germination by the van Tieghem hanging drop technique. All substances tested, except lactoflavin (vitamin B_2 or G), were synthetic crystalline preparations. The lactoflavin preparation was obtained from natural sources of lactoflavin and probably contained appreciable quantities of impurities carried over from the original material. A synthetic crystalline preparation of lactoflavin was obtained after the completion of most of the experiments reported herein, and was tested for its effect on germination.

Each experiment contained a distilled water control which invariably showed less than 5 per cent germination. TRAUB and O'RORK (**8**) have shown that papaya pollen germinates satisfactorily in a 0.75 per cent agar and 4 per cent sucrose medium. Accordingly this medium was carried along as a check in some of the experiments, in order to compare the present results with those of the past. Agar was not mixed with the substances being tested because it is a natural product consisting of a mixture of substances.

In the inoculation of the hanging drop in the spore experiments, a small number of spores were carried over from sterile cultures on the point of a needle. Distilled water controls did not germinate if care was exercised to avoid carrying over any of the substratum during the inoculation process.

A preliminary survey of the relation of time to percentage germination in the test solution showed that maximum germination of pollen was nearly always obtained after 4 hours and maximum germination of spores after 20 hours. Accordingly these time intervals were used uniformly throughout the investigation.

Investigation

POLLEN OF CARICA PAPAYA

It has recently been reported by DANDLIKER, COOPER, and TRAUB (**3**) that the addition of thiamin (vitamin B_1) to the Traub and O'Rork sugar-agar media causes a slight increase in the percentage germination of pollen of the Florida and Fairchild varieties of *Carica papaya*, as compared with that obtained on sugar agar without the addition of B_1. No increase, however, was obtained for the Orlando variety. Vitamin B_1 has now been tested without the sugar-agar

supplement and has been found to have some activity (tables 1 and 2). Just as with the earlier experiments, the maximum response was

TABLE 1

SUBSTANCES FOUND ACTIVE IN INDUCING GERMINATION OF ORLANDO PAPAYA POLLEN

SUBSTANCE	EFFECTIVE CONCENTRATION (GAMMA PER CC.)		MAXIMUM PERCENTAGE GERMINATION*			
	MIN.	MAX.	OCT. 11	OCT. 13	OCT. 15	OCT. 24
Distilled water	.....	.....	0	2	0	0
Lactoflavin† (vitamin B_2 or G)	10	50	82	78	74	67
Ascorbic acid (vitamin C)	50	100	96	89	55	56
Thiamin (vitamin B_1)	25	100	11	15	10	.....
Nicotinic acid	75	100	4	12	11	.....
Indole(3)acetic acid	25	100	37	8	33	7
Histidine-hydrochloride	50	200	23	41	40	.....
Cysteine-hydrochloride	50	100	12	49	45	.....
Glutamic acid hydrochloride	50	100	.....	28	30	.....
Lysine-dihydrochloride	50	200	45	.....	35	.....

* Averages of four determinations.
† Natural preparation.

TABLE 2

EFFECT OF DIFFERENT MEDIA ON GERMINATION OF POLLEN OF FOUR VARIETIES OF PAPAYA

VARIETY	PERCENTAGE GERMINATION IN MEDIA* (AVERAGE OF EIGHT DETERMINATIONS)					
	WATER	THIAMIN	LACTOFLAVIN†	ASCORBIC ACID	INDOLE(3)-ACETIC ACID	0.75% AGAR PLUS 4% SUCROSE
Orlando	3	10	78	76	17	49
Florida	0	52	78	62	39	61
Fairchild	0	12	75	72	12	71
Betty	0	35	64	56	7	.....

* Concentration 100 gamma per cc. in all cases except lactoflavin which was 50 gamma per cc.
† Natural preparation.

obtained with the Florida variety and the minimum with the Orlando variety.

In addition to thiamin, a number of other substances were found to be active in inducing germination. Table 1 shows that lactoflavin (natural product) and ascorbic acid were highly effective. Both showed considerably higher percentage germination than thiamin or any of the other substances tested. Also the percentages obtained were higher than those for the sugar-agar medium (table 2). The maximum and minimum effective concentrations of lactoflavin

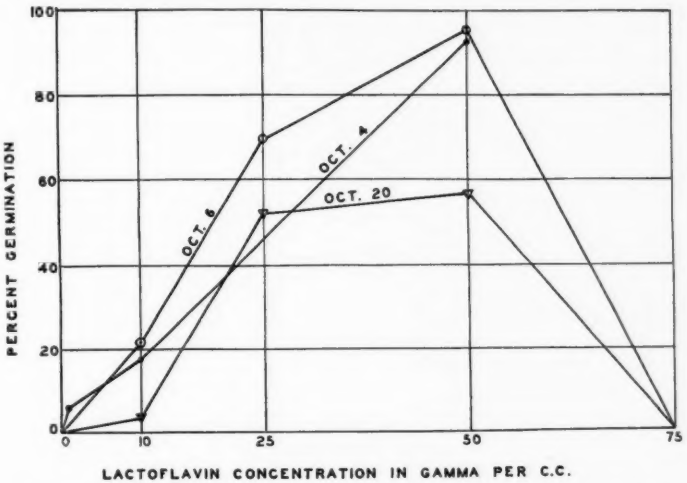

FIG. 1.—Effect of lactoflavin (natural product) on germination of pollen of *Carica papaya* var. Orlando (Hort.).

were 10 and 50 gamma per cc. and those of ascorbic acid were 50 and 100 gamma per cc. (figs. 1, 2).

It is noted from table 2 that lactoflavin and ascorbic acid caused high germination percentages for all varieties tested, that of the Orlando variety being as high as or higher than that for the Florida variety. Thus the lactoflavin and ascorbic acid reactions differ from the thiamin reaction, not only in giving greater germination but also in giving nearly equal germination for the various varieties.

The other compounds listed as being effective in inducing germination include nicotinic acid, indole(3)acetic acid, histidine hydrochloride, cysteine hydrochloride, glutamic acid hydrochloride, and

lysine dihydrochloride. It is seen that the amino acids in this group are all hydrochlorides. Other amino acids such as leucine, arginine, valine, asparagine, tryptophane, glycine, alanine, and tyrosine which contain a free NH_2 group were found to be practically inactive under the conditions of these experiments.

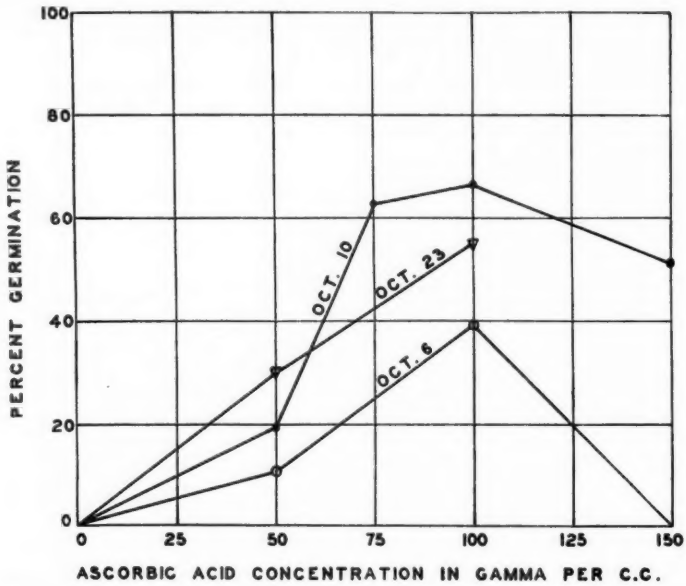

FIG. 2.—Effect of ascorbic acid on germination of pollen of *Carica papaya* var. Orlando (Hort.).

All these observations suggested that the activity of this group of compounds was caused by a pH effect and that any acid might produce the same effect. This was found to be the case when germination percentages comparable with those obtained by indole(3)acetic acid and the amino acid hydrochlorides were obtained with hydrochloric acid. Acetic, benzoic, and malic acids were also found to be slightly active.

The fact that substances in the first group (lactoflavin and ascorbic acid) gave considerably higher and somewhat more regular germination percentages than the substances of the second group would suggest that these two substances may possibly exert a greater

specific effect on germination than that of influencing the pH. It is possible that the lactoflavin preparation, which was a natural product, contained some impurity that was active in inducing germination. A 500 gamma per cc. solution of the natural product was analyzed spectroscopically[1] and found to contain 1.7 gamma per cc. of boron and traces of aluminum, iron, manganese, and magnesium. It has been reported in recent years that boron is essential to the germination of many kinds of pollen (**6**). Boron in the form of sodium borate has been tested for its effect on papaya pollen and found to induce germination at concentrations ranging from 0.001 to 1 gamma per cc. It would appear that the response obtained from the natural lactoflavin preparation might be due in part to the presence of boron as an impurity.

Sometime after the completion of these experiments, a synthetic preparation of lactoflavin was tested for its effect on pollen germination and was found to be active. It was, however, only about half as active as the natural product. Apparently lactoflavin does induce pollen germination, but the response obtained from the natural preparation might have been due to a combined effect of lactoflavin and boron.

As a matter of record it is well to mention that carotene (provitamin A), vitamin B_6, and a-tocopherol (vitamin E) were tested for their effect on pollen germination and were found to be without effect. Also sucrose, which is a constituent of most pollen germination media, was found in these experiments to be without activity for 1, 2, 5, 10, and 20 per cent solutions. Sucrose added as a supplement to lactoflavin caused no increase in percentage germination but caused a considerable increase in the length of the pollen tubes.

Spores of Colletotrichum gloeosporioides

Preliminary experiments on the effect of the various substances on germination of spores of *Colletotrichum gloeosporioides* gave very conflicting results. The first trial showed that thiamin was effective; a second and third trial showed that it had no effect, while lactoflavin (natural product) was quite active in inducing germination. These conflicting results were understood when it was found that the na-

[1] Spectroscopic analysis was made by Dr. B. C. Brunstetter of the U.S.D.A. Horticultural Research Station, Beltsville, Md.

ture of the growth medium from which the spores were taken influenced materially the response induced by various germinating media. When spores were taken from cornmeal-agar cultures, lactoflavin and a number of other substances, including thiamin, nicotinic acid, ascorbic acid, carotene, a-tocopherol, malic acid, inositol, pimelic acid, tryptophane, leucine, glycine, asparagine, arginine, glutamic acid hydrochloride, and histidine hydrochloride, would induce germination. On the other hand, when spores were taken from oatmeal-agar cultures, only lactoflavin was effective in inducing germination. Thus in the first preliminary experiment thiamin was active in inducing germination because the spores were taken from cornmeal-agar cultures, while in the second and third experiments thiamin was not active because the spores were taken from oatmeal-agar cultures.

The minimum effective concentration of lactoflavin on spores from oatmeal-agar cultures, under the conditions of these experiments, was about 100 gamma per cc., that concentration giving nearly 100 per cent germination while a 50 gamma per cc. solution gave practically no germination. Concentrations of thiamin, nicotinic acid, carotene, ascorbic acid, and several of the amino acids ranging from 0.1 to 500 gamma per cc., with and without sucrose, caused no germination of spores cultured on oatmeal agar. At the same time a concentration of 100 gamma per cc. of any one of these substances, with or without sucrose, would induce 50 to 100 per cent germination of spores cultured on cornmeal agar. These results were repeated again and again and held true for *Colletotrichum gloeosporioides* strains from both the avocado and the papaya.

Boron in concentrations ranging from 0.001 to 10 gamma per cc. was tested for its effect on the germination of spores grown on oatmeal agar and in no instance was any germination detected. Also, synthetic lactoflavin was found to be just as active as the natural product. This would more or less rule out an interpretation that the effectiveness of the lactoflavin preparation was due to the presence of boron as an impurity. A pH effect of lactoflavin is ruled out because malic and several other acids failed to effect germination of these oatmeal-cultured spores. The stimulation of the germination of cornmeal-cultured spores by malic acid might be a pH effect, but

that cannot explain the action of carotene, a-tocopherol, and a number of amino acids with free NH_2 groups. Also indole(3)acetic acid was not effective.

Spores of Penicillium italicum

Lactoflavin, thiamin, nicotinic acid, ascorbic acid, malic acid, carotene, and a-tocopherol were all found to be inactive in inducing the germination of *Penicillium* spores taken directly from an orange. These spores, however, were induced to germinate by addition of either leucine, cystine, cysteine hydrochloride, valine, tryptophane, and glutamic acid hydrochloride. Since amino acids with a free NH_2 group as well as the hydrochlorides were active, it appears that the limiting factor in this case is nitrogen in the amino form.

The addition of sucrose to the amino acid enhanced germination while the addition of lactoflavin inhibited it materially. Just why *Colletotrichum* spores need lactoflavin to germinate and *Penicillium* spore germination is inhibited by it is not known. It finds a parallel in the work of Schopfer (7), in which it was found that while many kinds of molds need B_1 to grow, *Rhizopus* is inhibited by it.

Summary

1. Lactoflavin (natural product) and ascorbic acid were both highly effective in inducing germination of papaya pollen. Synthetic lactoflavin was only about half as active as the natural product.

2. The natural preparation of lactoflavin was found to contain boron, and boron was found to induce pollen germination. From these results it is concluded that the action of the natural preparation of lactoflavin may be the result of a combined effect of lactoflavin and boron.

3. Nicotinic acid, indole(3)acetic acid, and the hydrochlorides of several amino acids were slightly active in inducing germination of pollen, but the action of these substances appeared to be a pH effect.

4. Thiamin, nicotinic acid, ascorbic acid, carotene, lactoflavin, and a number of other substances induced germination of *Colletotrichum gloeosporioides* spores grown on cornmeal agar, but only lactoflavin induced germination of spores grown on oatmeal agar.

5. Synthetic lactoflavin was just as active in inducing germination

of *Colletotrichum gloeosporioides* spores cultured on oatmeal agar as was the natural lactoflavin preparation.

6. Amino acids induced germination of *Penicillium italicum* spores, while addition of lactoflavin to the amino acids inhibited germination materially.

The writer is indebted to MR. H. E. STEVENS, plant pathologist of the U.S.D.A., Orlando, Florida, for many helpful suggestions during the course of the spore experiments.

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PHOTOPERIODIC RESPONSES OF DILL, A VERY SENSITIVE LONG DAY PLANT[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 501

KARL C. HAMNER AND AUBREY W. NAYLOR[2]

(WITH THREE FIGURES)

In the literature published since the discovery of photoperiodism in 1920 (1), the responses of many plants to photoperiod, whether long day, short day, or day neutral, are presented. The fact that a plant is listed as long day, or as short day, may not of itself be sufficient information on which to base the selection of any variety for critical experimentation. All short day plants do not respond with equal rapidity to short photoperiods, nor do they exhibit identical responses at different temperatures (5). The same is true for long day plants. Some varieties may be photoperiodically sensitive at one range of temperature and day neutral at another.

In previous papers (2, 3) it has been pointed out that attempts at integration of the effects of photoperiod on the sexually reproductive responses of plants should take into account the several stages in development of flower, fruit, and seed. *Xanthium pennsylvanicum* (3) will remain vegetative indefinitely if grown continuously under conditions of long photoperiod; if exposed to photoperiods which result in the initiation of floral primordia, these primordia develop into flowers and fruits under a wide range of photoperiod, even under continuous light. On the other hand, potato (4) will initiate floral primordia even under conditions of continuous darkness, but conditions of long photoperiod are favorable for the development of these primordia into flowers and fruits. There are so many other interrelations between phases of vegetative and sexually reproductive development and environmental conditions that at present any generalization should be made with great caution. A critical comparison of the responses of several plants to a variety of environmen-

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

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tal conditions is necessary before such generalizations can be made, and as yet sufficient information is not available to do this.

In connection with the experiments on *Xanthium*, it was desired to investigate the responses of some long day plant and to compare the two types. Many long day plants were investigated in a preliminary survey, including dill, aster (two species), *Scabiosa*, *Lepidium* sp., *Sisymbrium* sp., beet (biennial), wheat, and timothy, all of which have been reported as long day plants. Of all these, dill (*Anethum graveolens* Linn.) proved the most sensitive. The work reported here deals with some of its responses.

The seeds used throughout this work were from commercially available supplies and germinated well. They were planted in flats on a well lighted greenhouse bench under conditions of short (9-hour) photoperiod and transferred to $3\frac{1}{2}$-inch pots after the seedlings had developed from five to ten expanded leaves. As here used, the term long photoperiod refers to an exposure consisting of the normal daylight prevailing at any particular season of the year, supplemented when necessary by Mazda light of never less than 60 foot-candles intensity, giving a photoperiod of 18–19 hours' duration, alternating with dark periods of 6 or 5 hours' duration. Short photoperiod refers to exposure to 9-hour photoperiods alternating with 15-hour dark periods.

Investigation

In the spring of 1938, several hundred seedlings of dill were grown in the greenhouse under conditions of short photoperiod. About 2 per cent of the young plants developed seed stalks in a short time and were discarded. Many of the remaining plants were kept in the greenhouse all summer until the spring of 1939, being exposed throughout this period to conditions of 9-hour photoperiods. Except for the few plants which flowered shortly after planting, all remained strictly vegetative and in the rosette condition. During the spring, summer, and fall there were relatively wide variations in greenhouse temperatures, at times ranging as low as 50° F. and at others as high as 100° to 110° F. From our stock of seed, at least 98 per cent of the plants were strictly long day and remained vegetative indefinitely under conditions of 9-hour photoperiods, even under wide ranges of temperature.

In preliminary work it was found that dill plants placed on long photoperiod (18–19 hours) flowered within 5 weeks, even though the plants were very young and possessed only three to four expanded leaves. Plants which received ten long photoperiods and were subsequently grown under conditions of short photoperiod flowered approximately as soon as comparable plants maintained continuously on long photoperiod. To supplement these preliminary results, the following experiments were carried out.

On January 7, fifty strictly vegetative plants were transferred from conditions of short photoperiod to conditions of long photoperiod and each day thereafter five plants were returned to conditions of short photoperiod. Thus some plants were exposed to one long photoperiod only, still others to two long photoperiods, and so on, some plants receiving a total of ten long photoperiods. All plants which had been subjected to four or more long photoperiods produced a flowering stalk and flowered when returned to and maintained on short photoperiod. One of the plants receiving three long photoperiods flowered while the other four plants did not. None of those plants receiving but one or two long photoperiods flowered. These latter plants were continued on short photoperiod until March 15, at which time some of them showed some stem elongation although flower primordia could not be distinguished. Evidently dill plants may be photoperiodically induced by exposure to four long photoperiods.

Another experiment was carried out in a slightly different manner. Eighty-eight vegetative plants were selected from among those on short photoperiod on February 25. Seventy-two of them were transferred to conditions of continuous illumination while sixteen were continued on short photoperiod to serve as controls. The plants on continuous illumination were exposed to natural daylight for 9 hours, and to more than 60 foot candles of light from a Mazda lamp for the remaining 15 hours of each day. After 36 hours of such continuous illumination, sixteen plants were returned to and maintained on short photoperiod. Sixteen more plants were returned to short photoperiod after 60 hours' exposure, sixteen more after 84 hours, sixteen after 108 hours, and sixteen after 132 hours. The results are summarized in table 1. It may be possible that some of the plants

receiving 36 or 60 hours of continuous illumination would have had floral primordia if they had been harvested at a later date, since all of them showed some stem elongation, but the plants were harvested on March 28 because some of them had just reached the flowering stage on that date and maximum differences could be noted between the various treatments (fig. 1).

TABLE 1

EFFECT OF CONTINUOUS ILLUMINATION OF VARYING DURATION UPON STEM ELONGATION AND FLORAL INITIATION AND DEVELOPMENT IN DILL. BEFORE AND AFTER PERIODS OF CONTINUOUS ILLUMINATION, ALL PLANTS WERE MAINTAINED ON SHORT PHOTOPERIODS. VARIOUS TREATMENTS STARTED FEBRUARY 25; OBSERVATIONS MADE MARCH 28

	NUMBER OF HOURS OF CONTINUOUS ILLUMINATION USED IN ANY ONE TREATMENT					CONTROLS CONTINUED ON 9 HOUR PHOTOPERIOD
	132	108	84	60	36	
Average length (in cm.) of stem, based on 16 plants	57.3	38.9	19.1	19.0	8.1	3.3
Floral condition based on 16 plants...	16 with macroscopic flowers	16 with macroscopic flowers	14 with macroscopic flowers; 2 vegetative	6 with inflorescence primordia; 10 vegetative	2 with inflorescence primordia; 14 vegetative	16 vegetative

Perhaps the most striking differences between the various treatments are found in the relationship between the number of hours of continuous light the plants received and the amount of stem elongation. Up to 132 hours of treatment, the amount of stem elongation is almost directly proportional to the number of hours of light (with the exception of the 60-hour treatment which has a value somewhat high).

Experiments were conducted to determine whether or not, in the case of dill, the perception of the photoperiodic stimulus was received by the leaves. In a representative experiment, six pots of plants were placed in each of two wooden boxes of such depth that the

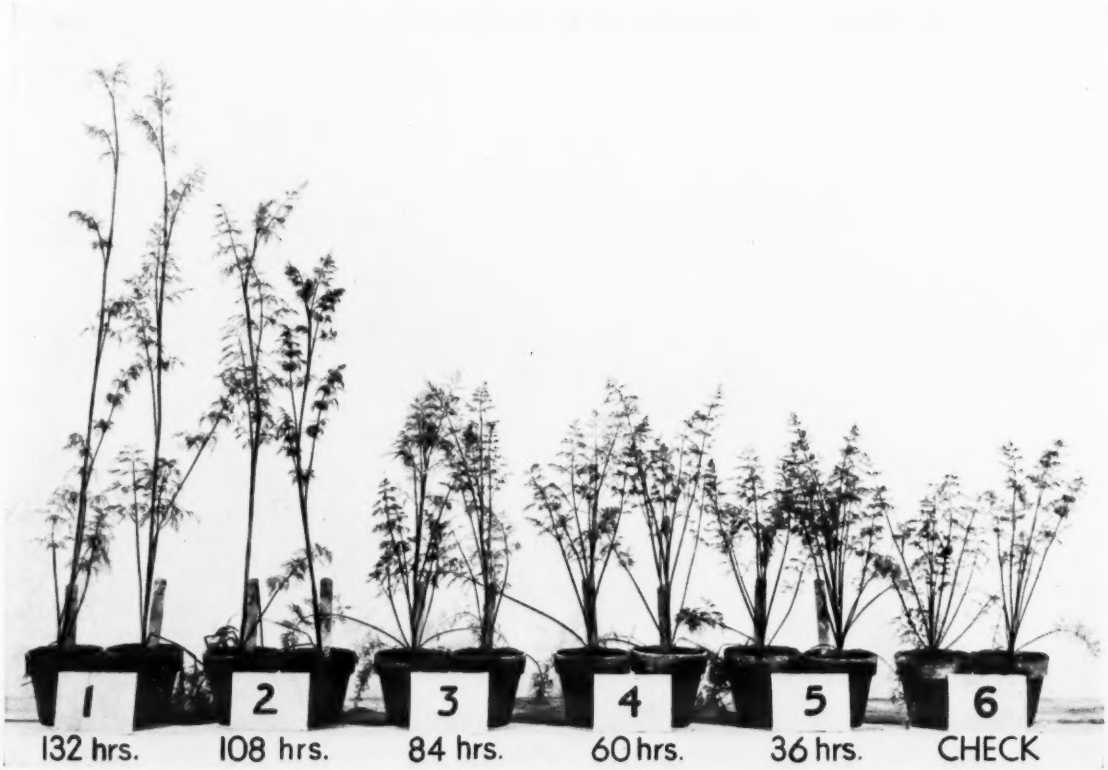

FIG. 1.—Response to continuous illumination for varying lengths of time: *1–5*, beginning February 25, plants received continuous illumination in excess of 9 hours as indicated by numbers; *6*, controls maintained on 9-hour photoperiod only. Before and after the indicated periods of continuous illumination, plants of *1–5* were maintained on 9-hour photoperiods. Photograph taken March 28.

leaves of the plants partially extended up out of the open top of the box. These boxes were painted black on the inside and were light-tight except for the top, which was open. Deep notches were made

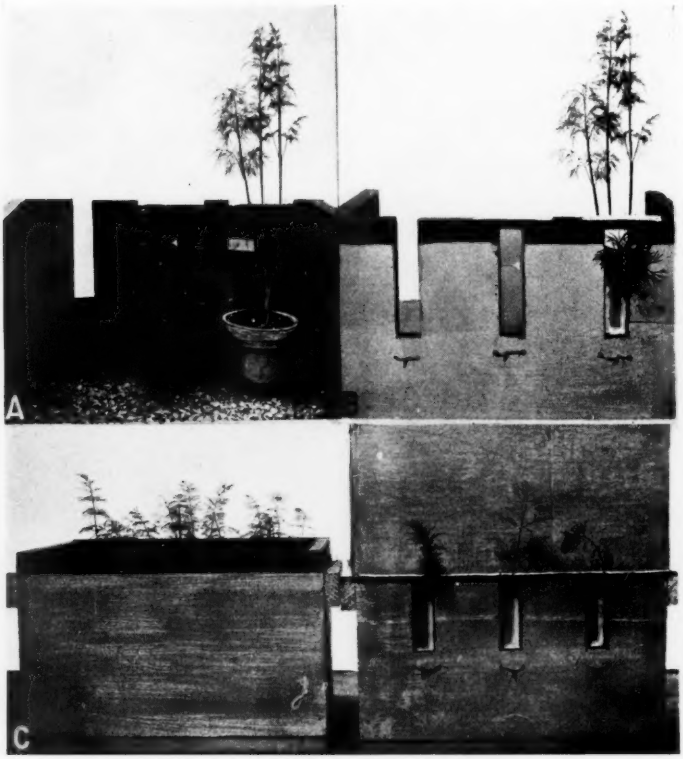

FIG. 2.—Apparatus used to subject entire plants or parts of plants to same or different photoperiods at same time. *A*, showing slit in box, covered by cardboard tacked in place, and plant in position; *B*, outside view showing same assembly; *C*, control without inverted top and box in place to give short photoperiod to all but single leaf which remains exposed to light.

in the sides of one box and one leaf from each plant extended out of the box through the notch (fig. 2). After each leaf was in position, a piece of notched cardboard was placed over the notch in the box so that the petiole of the leaf extended through both notches. The

notch in the cardboard was placed at right angles to the notch in the box and the cardboard was then tacked in place. The small space next to the petiole where light might still enter the box was sealed with a mixture of modeling clay and castor oil. Thus all the leaves of each plant, with the exception of the one, were inside. The other

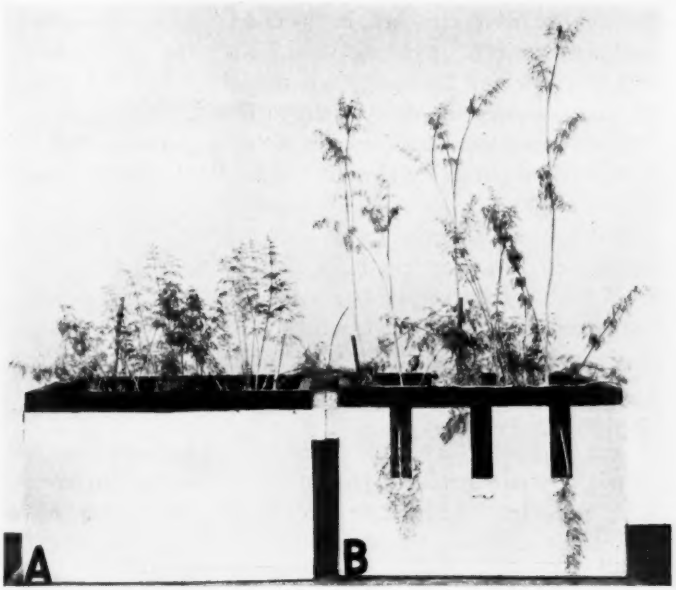

FIG. 3.—Effect of exposure of one leaf to long photoperiod while remainder of plant received short photoperiod. *A*, control, all leaves exposed to short photoperiod only; *B*, one leaf of each plant received a long photoperiod (January 14 to February 25).

box contained control plants and all the leaves of these plants were inside. The two boxes were placed under conditions of long photoperiod, and each night at 5:00 P.M. supplementary boxes were inverted over those containing the plants, so that no light reached any of the leaves of the various plants except the one leaf of each plant extending outside as described. At 8:00 A.M. the supplementary boxes were removed. Thus one leaf on each of six plants was exposed to conditions of long photoperiod while the remainder of the

plant was exposed to conditions of short photoperiod. At the same time six other comparable control plants had all their leaves exposed to short photoperiod only. This experiment was repeated in its entirety three times.

In every case, all plants having one leaf exposed to long photoperiod flowered within 6 weeks after start of the treatment. None of the control plants on short photoperiod flowered even though the experiment was continued for several months (fig. 3). Thus in dill, which is a long day plant, exposure of a single leaf to long day is sufficient to induce the entire plant to flower. In this respect dill somewhat resembles *Xanthium*, a short day plant, which will flower provided but a single leaf on a given plant is exposed to short photoperiod.

A number of other experiments were conducted, designed to determine the critical day length for dill. These experiments demonstrated that vegetative plants remain vegetative under ordinary greenhouse conditions at photoperiods less than 11 hours' duration and always flower at photoperiods in excess of 14 hours. Perhaps this lack of a sharp critical period in dill may be attributed to lack of genetic uniformity.

Under the conditions here described, dill is a long day plant which responds very rapidly to long photoperiod by coming into bloom. It has a short induction period, is easily grown under ordinary greenhouse conditions, and if subjected to short photoperiods will not be induced to flower by temperature variations ordinarily encountered in greenhouse work.

Summary

1. Approximately 2 per cent of the seedlings from the commercial dill seed employed flowered soon after germination if grown on 9-hour photoperiods, while the remainder continued in a rosette condition for 11 months when grown in the greenhouse at the same photoperiod.

2. Vegetative plants transferred from conditions of short to those of long photoperiod were induced to flower after exposure to as few as four long photoperiods.

3. If vegetative plants which have been maintained on short

photoperiod are transferred to continuous light for a period up to a total of 132 hours and then returned to short photoperiod, the rate of subsequent stem elongation is roughly proportional to the number of hours of continuous light received.

4. The critical photoperiod of dill, under ordinary greenhouse conditions, is between 11 and 14 hours.

5. Exposure of a single leaf to long photoperiod, although the remainder of the plant is kept on short photoperiod, is sufficient to induce the entire plant to flower.

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DEVELOPMENT OF MEGAGAMETOPHYTE IN ERYTHRONIUM ALBIDUM[1]

D. C. COOPER

(WITH SIXTEEN FIGURES)

Introduction

When it appeared that the development of the megagametophyte in *Lilium* (4, 5, 6) and *Tulipa* (3) is similar to that in *Fritillaria* (1, 2), material of *Erythronium albidum* Nutt., another member of the tribe Tulipeae of the Liliaceae, was collected for examination and comparison. SCHAFFNER (13) had found that the egg is removed from the macrospore mother cell by three divisions rather than by four, as has been described later for the genera first mentioned.

The material was collected from plants growing in an area of open woods on the campus of the University of Wisconsin. Ovaries from buds of various ages, as well as from open and withered flowers, were cut transversely into small pieces and either fixed in Carnoy's solution (3 parts 95 per cent alcohol, 1 part glacial acetic acid) or dipped in that solution and then placed in Karpechenko's modification of Navashin's fluid. Satisfactory preparations were obtained by both methods. The ovaries were sectioned transversely at 15 to 18μ, stained in dilute Delafield's haematoxylin, and counterstained with safranin.

Observations

The primary archesporial cell of *Erythronium albidum* functions as the macrospore mother cell. This cell is approximately three times as long as broad at the time of the first meiotic equatorial plate, and is somewhat flattened longitudinally. The heterotypic spindle lies in the mid-portion of the cell, its axis coinciding with the longitudinal axis (fig. 1). The spindle is completely surrounded by a dense layer of cytoplasm. The remaining cytoplasm of the cell is finely.

[1] Papers from the department of botany and the department of genetics (no. 238), Agricultural Experiment Station, University of Wisconsin. Published with the approval of the director of the station.

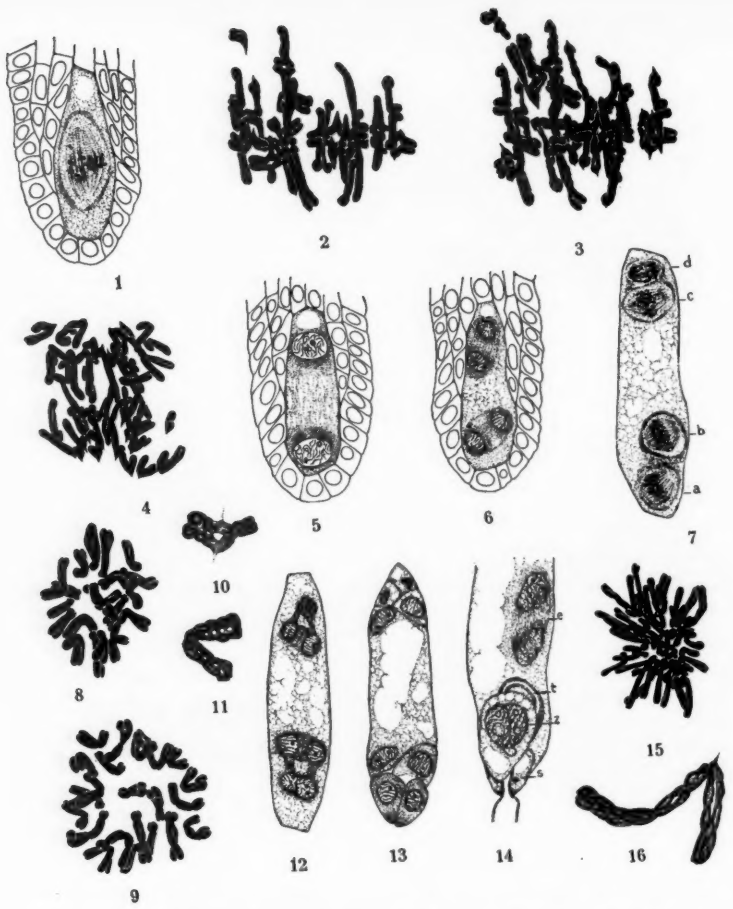
vacuolate, except that the chalazal region is occupied by a large vacuole.

Twenty-two pairs of chromosomes are present at diakinesis and on the heterotypic equatorial plate (figs. 2, 3). Anaphase figures show 22 chromosomes passing to each pole (fig. 4). The chromosomes vary in size and shape and in the position of the spindle fiber attachment. The two nuclei formed as a result of the heterotypic division are located one at each end of the mother cell. They pass into a prolonged resting stage and the heterotypic spindle disintegrates, the central region of the cell becoming more or less vacuolate. During this interphase period a zone of dense cytoplasm surrounds each of the nuclei (fig. 5).

The spindles of the homoeotypic division lie at oblique angles to the longitudinal axis of the cell. As a result of this division four nuclei are formed (fig. 6). The homoeotypic spindles are more or less persistent. In the interim between this and the third and last division, the cell grows and elongates to such an extent that it is fully four times as long as broad, and the cytoplasm in its mid-region becomes highly vacuolate.

Three of the four nuclei, the two at the micropylar end of the cell (fig. 7*a*, *b*) and the one at the chalazal end nearest the central vacuolated region (fig. 7*c*), undergo typical mitoses. Twenty-two chromosomes are present on each equatorial plate. Figures 8 and 9 are polar views of the chromosomes on equatorial plates located, respectively, in positions *c* and *a* (fig. 7). The nucleus nearest the chalazal end of the sac (fig. 7*d*) enters upon a more or less abortive division. Masses or aggregations of chromatic material, rather than definite chromosomes, appear on the spindle. Portions of this material pass irregularly to each pole and two irregularly shaped nuclei are formed simultaneously with the development of normal nuclei in other portions of the megagametophyte. Abortive divisions of the chalazal nucleus of a similar character have been figured and described (**12, 11, 5, 6**) for various species of *Lilium*.

Each spindle, during the third and last division in the megagametophyte, is surrounded by a dense layer of cytoplasm (fig. 7). Remnants of the spindles of the preceding division persist between the respective dense layers. The four nuclei at each end of the mega-



FIGS. 1–16.—Fig. 1, nucellus; macrospore mother cell at equatorial plate stage; ×175. Figs. 2, 3, heterotypic equatorial plates with 22 pairs of chromosomes; ×830. Fig. 4, heterotypic anaphase; ×830. Fig. 5, nucellus; 2-nucleate megagametophyte; ×175. Fig. 6, nucellus; 4-nucleate megagametophyte with persistent homoeotypic spindles; ×175. Fig. 7, equatorial plates; last (third) nuclear division in megagametophyte; ×175. Fig. 8, polar view of haploid equatorial plate, position *c;* ×830. Fig. 9, same, position *a;* ×830. Figs. 10, 11, chromosomes from haploid equatorial plates showing four chromonemata; ×1600. Fig. 12, 8-nucleate megagametophyte showing cell plate formation; ×175. Fig. 13, mature megagametophyte; ×175. Fig. 14, late stage in fertilization showing zygote (*a*), dividing primary endosperm nucleus (*e*), disintegrating pollen tube (*t*), and synergids (*s*); ×175. Fig. 15, polar view of diploid equatorial plate with 44 chromosomes; ×830. Fig. 16, chromosome from diploid equatorial plate with four chromonemata; ×1600.

gametophyte formed as a result of the final division are connected by two prominent spindles and by portions of a less prominent one (fig. 12), as described by SCHAFFNER (13). The synergids and egg at the micropylar end and the antipodal cells at the chalazal end are delimited as a result of cell plate formation across the spindles of the last division, as well as across the persistent spindles of the preceding division. The last division was taking place in ovaries taken from open flowers. In some instances pollen tubes were found in the region of the micropyle at the time the nuclei of the megagametophyte were in the third division.

A typical 8-nucleate, 7-celled megagametophyte is formed (fig. 13). SCHAFFNER described an 8-nucleate megagametophyte in which the egg apparatus was not well organized. GUERIN'S (8) description of the mature megagametophyte in *Erythronium dens canis* is similar to that herein described for *E. albidum* (9).

The definite organization of the megagametophyte persists for a short time only. If fertilization does not occur, the membranes delimiting the cells disintegrate and the eight nuclei lie free within the space in the nucellus formerly occupied by the gametophyte. Such aborting megagametophytes are present in many of the ovules in an ovary wherein some of the adjacent ovules have been fertilized. The polar nuclei and those of the egg apparatus increase greatly in size before disintegrating.

Fertilization occurs in the manner typical of most angiosperms. One male gamete nucleus unites with the egg nucleus; the other unites with the two polar nuclei. A late stage in fertilization is shown in figure 14, in which the fusing nuclei in the egg (*z*) are plainly evident. The collapsed pollen tube (*t*) lies beneath the zygote and the synergids (*s*) are in course of disintegration. The latter cells have become highly vacuolate and their nuclei are much shrunken. The primary endosperm nucleus (*e*) is in a late stage of the first division following fertilization.

The zygote, and at later stages the young embryo, remains at the micropylar end of the endosperm. No evidence has been found that either the zygote or the young embryo comes to lie free within the endosperm.

Discussion

SCHAFFNER (**13**) reported a haploid chromosome number in both *Erythronium albidum* and *E. americanum* as being 12, although his figure 68 of a homoeotypic equatorial plate seems to show 22. In the present study, 22 chromosome pairs were clearly seen at diakinesis and on the heterotypic equatorial plate. Polar views of equatorial plates at the time of the third division in the macrogametophyte showing 22 chromosomes (figs. 8, 9) and similar views of dividing nucellar nuclei with 44 chromosomes (fig. 15) confirm this number.

The chromosomes of dividing gametophytic nuclei (figs. 8, 9) fall into two types, based upon the region of spindle fiber attachment. Each chromosome of one type is V-shaped with the spindle fiber attachment region at or near its middle, whereas each one of the second type is a long shaft with a short rounded lobe beyond the attachment region. There are 8 chromosomes of the former type and 14 of the latter. Those of each type vary among themselves in length.

The equatorial plate chromosomes of dividing gametophytic nuclei are shorter and thicker than those at a similar stage in the adjacent nucellar tissue (fig. 15). Each chromosome on either a haploid (figs. 10, 11) or a diploid (fig. 16) equatorial plate consists of four chromonemata twisted about one another in pairs. This type of chromosome structure has been described in a number of plants which have large chromosomes (**7, 9, 10, 14,** and others).

The presence of the haploid number of chromosomes on one of the chalazal spindles (fig. 7*c*) of the last nuclear division in the formation of the megagametophyte, and the fact that the megagametophyte is removed from the spore mother cell by three rather than by four divisions, justify the reference of megagametophyte development in *Erythronium albidum* to the "Adoxa" type.

Summary

1. The primary archesporial cell of *Erythronium albidum* functions as a macrospore mother cell which develops directly into the megagametophyte.

2. In consequence of the two meiotic divisions followed by a single mitotic division and this by cell division, an 8-nucleate, 7-celled megagametophyte is formed.

3. The last mitotic division in the formation of the megagametophyte occurs just before fertilization.

4. Cell division is by means of cell plates.

5. The chromosome number is $n = 22$, $2n = 44$.

6. Eight V-shaped chromosomes with median spindle fiber attachments and 14 rodlike chromosomes with subterminal spindle fiber attachments are present in gametophytic nuclei.

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EFFECT OF CERTAIN GROWTH SUBSTANCES ON INFLORESCENCES OF DATES

ROY W. NIXON[1] AND F. E. GARDNER[2]

(WITH ONE FIGURE)

Varying degrees of fruit development have been induced in a number of plant species by the use of growth substances (1, 2). Attempts in 1938 to influence the course of development of date ovaries by means of these compounds were chiefly unsuccessful. A response of other portions of the inflorescence, however, appears to warrant brief mention.

Seedless fruits of *Phoenix dactylifera* often develop when pollination is prevented by bagging. As compared with pollinated seed-containing fruit, the unpollinated seedless fruit is usually smaller, somewhat different in shape, develops more slowly, and the occasional fruit that eventually ripens is generally rather fibrous and lacking in quality, although apparently of a sugar content comparable on a dry basis with the pollinated fruits (3). It was thought that treatment of flowers with growth substances might stimulate parthenocarpic fruits to a development more comparable with that of pollinated dates, and that, applied in conjunction with pollen, it might affect the set or the time of fruit ripening.

Indoleacetic, indolebutyric, and naphthaleneacetic acids were applied, alone and in combination with pollen, both before and after the application of the latter. The highest concentration used was 1.0 per cent in lanolin paste. Aqueous solutions between 0.1 and 0.01 per cent were applied as a spray, with further dilutions of naphthaleneacetic acid down to 0.001 per cent. Naphthaleneacetic acid was also applied as a dust mixed with talc, in concentrations from 1.0 to 0.01 per cent.

The higher concentrations of indoleacetic and indolebutyric acids not only failed to stimulate development of parthenocarpic fruits but resulted in the shedding of practically all flowers within a few weeks.

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In the lower concentrations there was less shedding of flowers, but the resulting fruits were apparently no different from those without

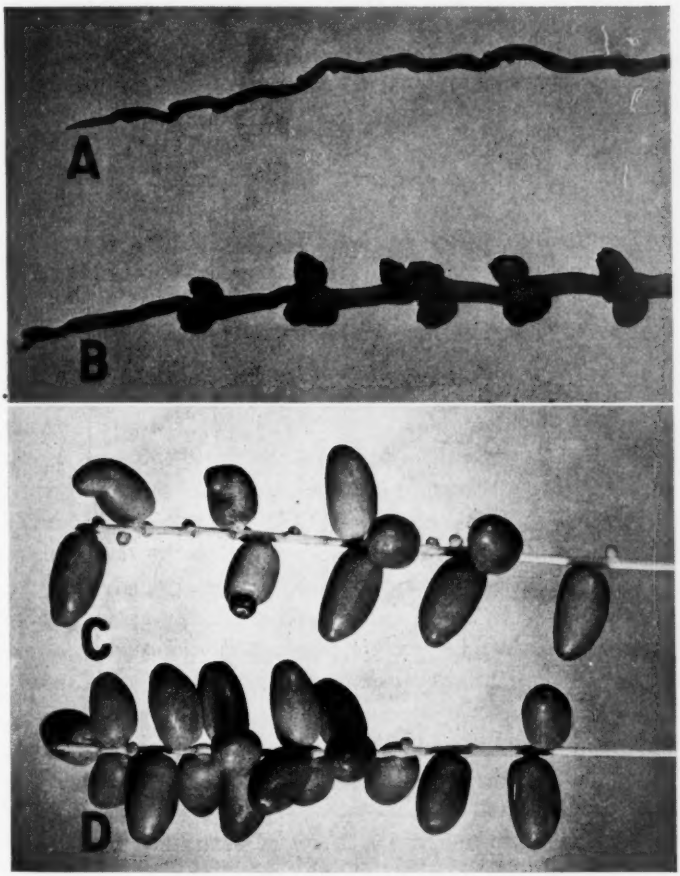

FIG. 1.—Effect of growth promoting substances of inflorescences of dates

growth substance treatment. Naphthaleneacetic acid in dust form was ineffective. In lanolin paste and in the higher concentrations of aqueous solutions, however, naphthaleneacetic acid inhibited development of the carpels, which eventually became dry and spongy,

but at the same time caused the enveloping perianths to persist and even to show apparent enlargement in some instances.

Figure 1*B* shows the effect of 1 per cent naphthaleneacetic acid in lanolin paste applied to date flowers of the Thoory variety on March 30, 1938. When cut on November 29 this strand with its persisting perianths was still alive and normally colored, except where the tip carrying two or three perianths had withered slightly. Much earlier than this, normally pollinated dates were all past ripe, with strands dead and dry. On the same bunch, strands treated with the other two chemicals in a similar manner lost all flowers and were dead and dry early in the season (fig. 1*A*). The results show the arrested senescence of the perianth and strand, an effect shown to occur in certain fruit tissues from growth substance application (**5**).

Figure 1*C* shows the effect on Deglet Noor dates, khalal stage, of naphthaleneacetic acid applied as an aqueous spray about ten days after pollination, first in concentration of 0.01 per cent and six days later in concentration of 0.02 per cent, as compared with untreated pollinated dates (fig. 1*D*). The set was reduced about 50 per cent by this treatment. Although less marked than in the higher concentrations, there was the same tendency toward restriction of carpellary development with persistence of perianths. Most of the pollinated dates that developed after naphthaleneacetic acid treatment appeared in every way like the untreated pollinated dates on adjacent bunches. A considerable number, however, developed with varying degrees of malformation as illustrated. The nature of the malformation indicates a marked injurious effect of the acid on the succulent carpellary tissue. Apparently unaffected dates among those treated ripened a little earlier and with a higher percentage of drier fruit than occurred on the untreated bunches. This, however, cannot be attributed directly to the growth substance but rather to the consequent reduced set, for it has been observed that severe thinning produces similar results (**4**).

The observations on the application of growth substances to inflorescences of dates emphasize the marked influence often exerted by these compounds, particularly by naphthaleneacetic acid, in ar-

resting senescence and delaying the abscission of various plant organs.

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CURRENT LITERATURE

Fortschritte der Chemie organischer Naturstoffe. Edited by L. ZECHMEISTER. Vienna: Springer, 1938. Pp. vi+371. Figs. 41. Rm. 28.

In the field of organic chemistry, scientific progress is so rapid that, without the ever-growing volume of reviews and monographs, the flood of publications would present a bewildering complexity of results. The *Fortschritte* is apparently to be an annual, of which this is the first volume. It is sponsored by some of the best organic chemists of Europe, including, in addition to the editor, BUTANANDT, KÖGL, HAWORTH, and SPÄTH. These names guarantee the quality of the reviews.

This volume contains seven reviews. The first, by ZEMPLÉN, deals with recent methods of glucoside syntheses' and describes seven methods of synthesizing glucosides: alkyl glucoside syntheses from free sugar and alcohol by chemical agents; biochemical syntheses; syntheses from acetohalogen compounds; transformations of β-glucosides into the α-form; mercury salt methods; preparation of biosides of the α-series without employment of acetohalogens; and Helferich's phenol-bioside syntheses with zinc chloride or p-toluol-sulphonic acid.

The second review, by HILDITCH, considers the component glycerides of vegetable fats. Some of the points covered are the isolation of individual triglycerides by crystallization; crystallization of halogenated fats; and quantitative studies of component glycerides. There is an important discussion of the glyceride structure of seed fats, of both liquid and solid types.

The reviews of recent advances in the chemistry of the sterols, by HEILBRON and SPRING, and of cozymase, by SCHLENK and VON EULER, are timely. Advances in these fields have been exceptionally rapid, and the reviews are a revelation of the insight into the structure of complicated organic cellular agents which organic chemistry has given us. Nucleic acid chemistry is reviewed by BREDERECK, the main consideration being given to the structure of the nucleosides, nucleotides, and polynucleotides. A review of chlorophyll, by STOLL and WIEDEMANN, occupies nearly 100 pages, and carries a literature list of over 400 references. It is a thorough going, masterful summary of current knowledge of this pigment and its derivatives.

The final review, by KRATKY and MARK, considers several topics, but mainly the use of physical methods in the investigation of natural substances, as regards the form and size of molecules, in the dispersed state. The determination of active groups, cryoscopy, vapor pressure depression, dialysis, osmometry, ultracentrifugation, and free diffusion are considered in the first section; in the second, the application of roentgenography to the study of structure is presented; the final section deals especially with the use of x-rays in the study of protein structure.

It is not possible here to do justice to this volume; it must be examined to grasp its significance. It is certainly one of the best groups of reviews on biochemistry issued in recent years. The series will be indispensable for all libraries and for all who are actively engaged in biochemical research.—C. A. SHULL.

The World of Plant Life. By C. J. HYLANDER. New York: Macmillan Co., 1939. Pp. xxiv+722. Illustrated. $7.50.

This survey was planned and written primarily for the layman as a "card of introduction to the outstanding personalities in the plant world. . . . . The natural interests of the average American, at home and vacationing in our summer and winter playgrounds, has been the criterion for the inclusion of material." It is secondarily designed for students as "an introduction to an alive, fascinating world which is often ignored in laboratory study"; and as a picture of the whole plant kingdom for amateur or professional specialists. The reviewer feels that all three aims have been fairly well achieved. Popularization with strict accuracy is not easy for most botanists, even in their special fields. Such treatment for the whole plant kingdom is an ambitious undertaking, and this one is deserving of commendation.

The author, recognizing the difficulty of his task, approached it from a taxonomic point of view. Upon the foundation of phylum, order, family, and tribe he has arranged interesting and accurate facts of form, function, ecology, distribution, and use of about 2000 of the more common native and introduced plants of continental United States. The emphasis is upon plants of the vacation centers of the northeast, Florida, California, and the southwest, and spectacular plants perhaps receive disproportionate attention. These features are understandable in a work of this kind. About three-fourths of the volume is devoted to angiosperms. The enjoyment of the book by both laymen and botanists is markedly increased by the numerous and beautiful photographic illustrations and clean and accurate pen-and-ink drawings. There is a good index, a well selected reference list, and a 47-page check list giving the scientific classification and common name of all species mentioned in the text. The book should rightly stimulate the interest of the general public in plants.—C. E. OLMSTED.

The Physiology of Plants. By WILLIAM SEIFRIZ. New York: Wiley & Sons, 1938. Pp. xvii+315. Figs. 95. $3.50.

A number of texts on plant physiology have appeared recently. This work by SEIFRIZ is characterized by its brevity and elementary nature. The entire field is covered in 26 chapters, in 305 text pages. Following a brief introduction, which defines the aims, scope, and historical background of the subject, the author gives early consideration to the colloidal state, soil, protoplasm, and the organized plant cell.

A fairly logical order of presentation follows, with water relations: osmosis, sap movement, use, loss, and storage of water the main consideration, in preparation for a study of the major processes. Acidity, salt requirements, and per-

meability follow in order, and then photosynthesis, respiration, carbon and nitrogen cycles, movement and storage of foods, nature of foods, and the products derived from them, such as cellulose, wall substances, etc., and other plant products. The later chapters discuss growth and hormones, plant cultures, reproduction, movements, plant associations, environment, and a chapter on the living and nonliving state. While the chapters are short, yet a great deal of information is provided. General principles are stressed, and the work will no doubt prove popular because it is not too technical nor too detailed.

The reviewer feels that the brevity of this text almost reaches the point of leaving inaccurate impressions; taken as a whole, the work corrects some of these impressions. On page 244, for instance, it is said that "water culture as a laboratory technique was known a quarter of a century ago." The implication seems to be that it began then; but the author has previously mentioned in another connection the early laboratory use of solution cultures of SACHS and KNOP, which are more than 60 years old. The experiment cited from HALES' work is called a root-pressure experiment, although the root is lifting a column of mercury under the influence of transpiration by the top of the tree. DUTROCHET'S discovery of osmotic movement of water was published originally in 1826, not in 1837. One may wonder also why the date 1838 is chosen as the starting point of plant physiology as a science.

Any slight faults which may be present are not likely to detract greatly from the usefulness of the book. There has been need of a work suitable for beginning students, and this text has caught something of the qualifications necessary for such a purpose.—C. A. SHULL.

Statistical Methods. By GEORGE W. SNEDECOR. Ames, Iowa: Collegiate Press, 1938. Pp. xiii+388. Illustrated. $3.75.

Those who may have found other works on statistical methods forbidding should examine this excellent exposition of the subject. It is addressed particularly to the novice, and the reviewer is impressed with the nontechnical language in which the various procedures are explained. The author has demonstrated that discussion of the mathematical principles involved in statistical studies is not necessarily uninteresting and unintelligible to the beginner. The more readily mastered principles and techniques are treated first, and new concepts are introduced one at a time; in this way progress is gradual, and each new step easy to take. Here is a manual of statistics which apparently can be used without excessive study of mathematics. It necessarily covers much the same ground as the well known work by R. A. FISHER, but the approach is somewhat simpler and less technical.

The first seven chapters deal with the simplest problems the investigator is likely to encounter: statistical study of attributes; comparative measurement of individuals; sampling of normal populations; statistical comparison of two groups; short cut methods and approximations; linear regression; and correlations. The succeeding chapters cover the more complicated cases: large sample

theory; multiple degrees of freedom; variance; covariance; multiple regression; curvilinear regression; individual degrees of freedom; and binomial and Poisson distribution.

Tables are introduced where they are needed, not in an appendix; and numerous examples from actual experience are used as problems to illustrate the processes of statistical analysis. This use of concrete material makes the presentation far more effective than would the mere discussion of principles and methods, however thorough.

The book is recommended as a constant companion of those who are attempting to establish the validity of conclusions from mass study of responses. Most investigators sooner or later find that statistics are necessary to the solution of their problems; moreover, if the principles are mastered, more frequent use would naturally be made of these methods of testing results. The plane of experimental work would be raised considerably if the statistical methods outlined in this volume were more generally employed. No better work is available for the beginner.—C. A. SHULL.

Hardy Bulbs, Vol. III: *Liliaceae*. By CHARLES HERVEY GREY. New York: Dutton & Co., 1938. Pp. x+664. Figs. 82. $11.00.

This volume is the third in a series in which an attempt has been made to describe—in a manner useful to gardeners and botanists alike—all bulbous, tuberous, and rhizomatous monocotyledons of garden value that are capable of cultivation in the open or in cool greenhouses, and to give information as to site, cultivation, and propagation.

Volume III embraces those plants which are classed as Liliaceous according to the system of ENGLER and PRANTL. The genera, numbering 110, are listed alphabetically. A brief generic description is given and this is followed by a description of the species which are considered as hardy and worthy of cultivation. The book is essentially a reference work and contains a vast amount of valuable information. It includes 19 color plates and 63 black and white drawings.—J. M. BEAL.

Experiments in Plant Physiology. By WALTER E. LOOMIS and CHARLES A. SHULL. New York: McGraw-Hill, 1939. Pp. xiv+213. $2.00.

This book contains directions for 167 elementary and intermediate experiments in plant physiology, for the most part selected from the more comprehensive text, *Methods in Plant Physiology*, published in 1937 by the same authors. The work covers practically every phase of the subject, and more experiments are presented than can be covered in the usual course. Some of the experiments can be performed with hand-made, inexpensive apparatus, while others require more elaborate and expensive equipment. The contents of the book are thus flexible so that it can be used in longer or shorter courses and by institutions that have little or much money for laboratory equipment.

The directions are admirably presented, in simple, clear, and concise lan-

guage so that the student may easily follow them. The book fills a long-felt need and is a distinct contribution to the subject of elementary plant physiology.—E. C. MILLER.

Hilfsbuch für das Sammeln und Präparieren der niederen Kryptogamen. 2d ed. By Dr. GUSTAV LINDAU; revised by Dr. O. C. SCHMIDT. Berlin: Borntraeger, 1938. Pp. vii+93. Rm. 3.60.

This revised booklet, which includes citations of a few 1938 United States publications, should be of value to botany students in general even though the bibliography is composed mainly of German publications.

While necessarily brief in its specialized part, which includes collecting directions for the various groups of thallophytes and bryophytes, this manual discusses in some detail many important or interesting genera groups and genera. Suggestions for collecting plants, and directions for drying them and preventing insect infestation are given; formulae for various preserving solutions are added where needed. The volume will be of greatest value to amateur collectors of Continental Europe, but may appeal to a wider, international audience.—P. D. VOTH.

Principles of Paleobotany. By WILLIAM C. DARRAH. Leiden, Holland: Chronica Botanica Co., 1939. Pp. 239. Plates 5.

Few universities offer a course in paleobotany, and most students who desire to become acquainted with this subject must depend upon literature and whatever museum collections are available. An introductory book in paleobotany is therefore a much desired addition to botanical literature. DARRAH'S arrangement of the subject is rather satisfactory. He gives the reader an idea of literature and technique before entering into the discussion of fossil plant phyla. As a whole, the morphological, ecological, and geological features are as well developed as the small space of the book permits. The beginner will find it interesting reading. The mature reader may differ with DARRAH on his valuation of events in paleobotanical history. It must also be regretted that a book which appeals primarily to the beginner should contain so few illustrations.—A. C. NOÉ.

Cotton. By HARRY BATES BROWN. 2d ed. New York: McGraw-Hill Book Co., 1938. Pp. xiii+592. Figs. 140.

This book brings up to date the material presented in a previous volume by the same author some twelve years earlier. There has been added considerable new material dealing with the morphology of the cotton plant, its culture, and the economic handling of the crop.—E. J. KRAUS.

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